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# Rapid Assessment of Water Quality, Using the Fingernail Clam, *Musculium transversum*

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AT URBANA-CHAMPAIGN
WATER RESOURCES
CENTER

APRIL 1978

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FINAL REPORT
Project No. B-097-ILL

This project was partially supported by the U.S. Department of the Interior in accordance with the Water Resources Research Act of 1965, P.L. 88-379, Agreement No. USDI 14-31-0001-6072

UNIVERSITY OF ILLINOIS
WATER RESOURCES CENTER
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April, 1978

## NOTE

The original title of this research project was "Rapid Assessment of Water Quality Using the Fingernail Clam, Sphaerium transversum". The scientific name of the clam was changed to Musculium transversum while the research was in progress. Some of the figures in this report use the older scientific name, Sphaerium transversum.

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## ABSTRACT

Apparatus and methods were developed for testing the effects of water quality factors on the ciliary beating rate of clam gills. Musculium transversum was chosen as the test organism because it is a major food source for fish and waterfowl, and because it has died out in areas of the Illinois River where it was formerly abundant. Populations of this fingernail clam also declined recently in the Keokuk Pool, Mississippi River, an important feeding area for migratory waterfowl and commercially valuable species of fish.

The ciliary beating response is extremely sensitive. For example, a zinc concentration of .00006 µg/l produced a statistically significant reduction in the ciliary beating rate of gills from large fingernail clams. Gills from small clams were much less sensitive, requiring .06-.6 µg/l zinc to produce the same response. Concentrations of potassium and un-ionized ammonia which inhibited the ciliary beating response of gills from small clams were quite close to the concentrations which reduced the survival or growth of intact clams during chronic bioassays. The threshold concentration of potassium for cilia inhibition of small clams lay between 39 and 390 mg/l. The maximum acceptable toxicant concentration (MATC) for long-term survival of fingernail clams lay between 195 and 275 mg/l potassium. Un-ionized ammonia concentrations of .08-.09 mg/l inhibited the cilia of small clams, and the growth of the clams was reduced at concentrations between .20 and .34 mg/l $NH_3$-N.

In addition to potassium and ammonia, the following factors were tested singly or in combination: light, temperature, dissolved oxygen, sodium nitrate, sodium sulfate, cyanide, lead, copper, zinc, suspensions of silica particles, suspensions of illite clay particles, and raw Illinois River water. Comparison of the levels of these water quality factors in the Illinois and Mississippi Rivers with the levels which had detrimental effects on the clam gills suggests that un-ionized ammonia and heavy metals may have affected fingernail clams in the Illinois River in the 1950's and in the Mississippi River in 1976-1977. These tentative conclusions should be validated using chronic bioassays in which fingernail clams are exposed to conditions simulating those in the Mississippi River in 1976-1977, and by deletion bioassays in which certain components are removed from raw Illinois River water and the survival, growth, and reproduction of clams in the treated water are measured.


KEY WORDS: water pollution effects, bioassay, bioindicators, animal physiology, fingernail clams, Sphaerium transversum, Musculium transversum, Sphaeriidae, heavy metals, silt, heat, dissolved oxygen, cyanide, ammonia, potassium, suspended solids, suspended sediment, sodium nitrate, sodium sulfate, lead, copper, zinc, Keokuk Pool, Mississippi River, Illinois River, Asiatic clam, Corbicula manilensis, blue mussel, Mytilus edulis, Elliptio complanata.

# INTRODUCTION AND BACKGROUND

Fingernail clams (Family Sphaeriidae) are dominant bottom-dwelling animals in some waters of the midwestern part of the United States. They are found in major rivers (Gale, 1969: v), in lakes (Emmling, 1974: 11), even in bottomlands which are only intermittently flooded (Hubert, 1972: 177-178), and are key links in food chains leading from nutrients in water and mud to fish and ducks which are utilized by man. Fingernail clams filter algae, bacteria, and organic matter from water. Because the clams are small (less than 15 mm long when full-grown), they in turn are readily consumed by ducks and bottom-feeding fish. A short food chain of this type can support a larger biomass at the top level (ducks and fish) than a longer one.

The fingernail clam, Musculium transversum (Say, 1829), shown in Figure 1, prefers big river habitat with a silt bottom where peak numbers may exceed 100,000/$m^2$ as in the Keokuk Pool, Mississippi River (Gale, 1969: v). Keokuk Pool is a 74-km (46-mile) section of the mainstem of the Mississippi extending from Lock and Dam 19 at Keokuk, Iowa to Lock and Dam 18 near Burlington, Iowa. Ranthum (1969) and Jude (1968, 1973) studied the food habits of fish in the Keokuk Pool and found that at certain times of the year fingernail clams made up 100% by volume of the diets of carp (Cyprinus carpio) and smallmouth buffalo (Ictiobus bubalus), and 10 to 70% of the diets of black bullhead (Ictalurus melas), gizzard shad (Dorosoma cepedianum), pumpkinseed (Lepomis gibbosus), bigmouth buffalo (Ictiobus cyprinellus), freshwater drum (Aplodinotus grunniens), and bluegill (Lepomis macrochirus). These fishes include commercial, sport, and forage species.

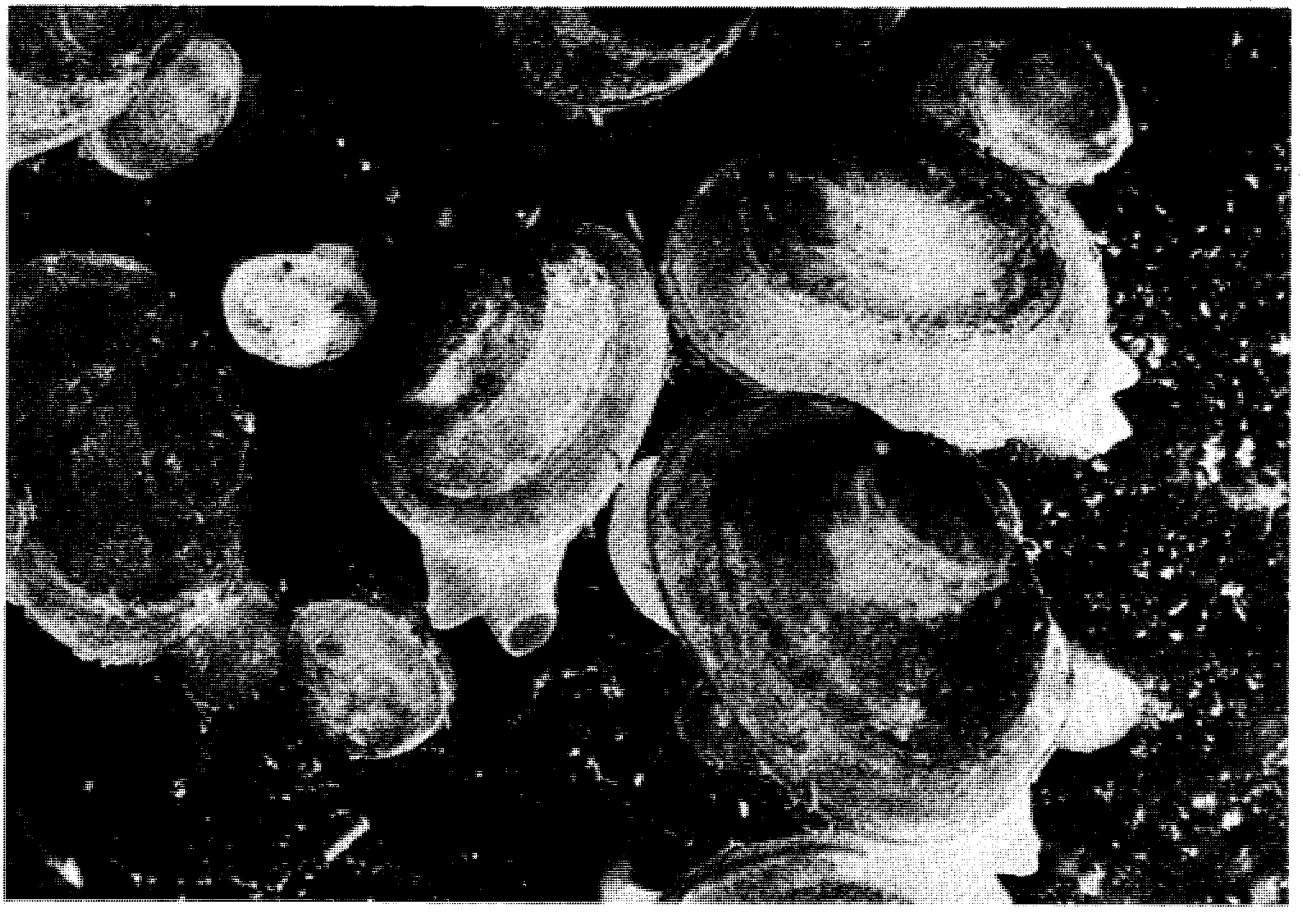


Figure 1. Live fingernail clams, Musculium transversum, which were raised in the laboratory. The large individuals are approximately 8 mm across the longest dimension of the shell, and will grow to 15 mm. The small, light-colored clam in the upper left is new-born and is approximately 2 mm across. The clam in the center has extended its siphons, and the individual just to the right and below center has extended its foot.

Thompson (1973: 379) estimated that lesser scaup ducks (Aythya affinis), ring-necked ducks (Aythya collaris), canvasbacks (Aythya valisineria), common goldeneyes (Bucephala clangula), and ruddy ducks (Oxyura jamaicensis) consumed 2.2 million kg (4.8 million pounds) of fingernail clams in Keokuk Pool during the fall migration of 1967, or approximately 24% of the standing crop of fingernail clams at the Keokuk Pool (Gale, 1973: 175). Thompson found that the clams made up 85-95% by volume of food items taken by these ducks in the spring of 1967. The Keokuk Pool attracts about 20 million diving duck-days of use per year (Personal communication, F.C. Bellrose, Wildlife Specialist, Illinois Natural History Survey, August, 1976) and has been characterized as the most important inland body of water for diving ducks in North America (Trauger and Serie, 1974: 71).

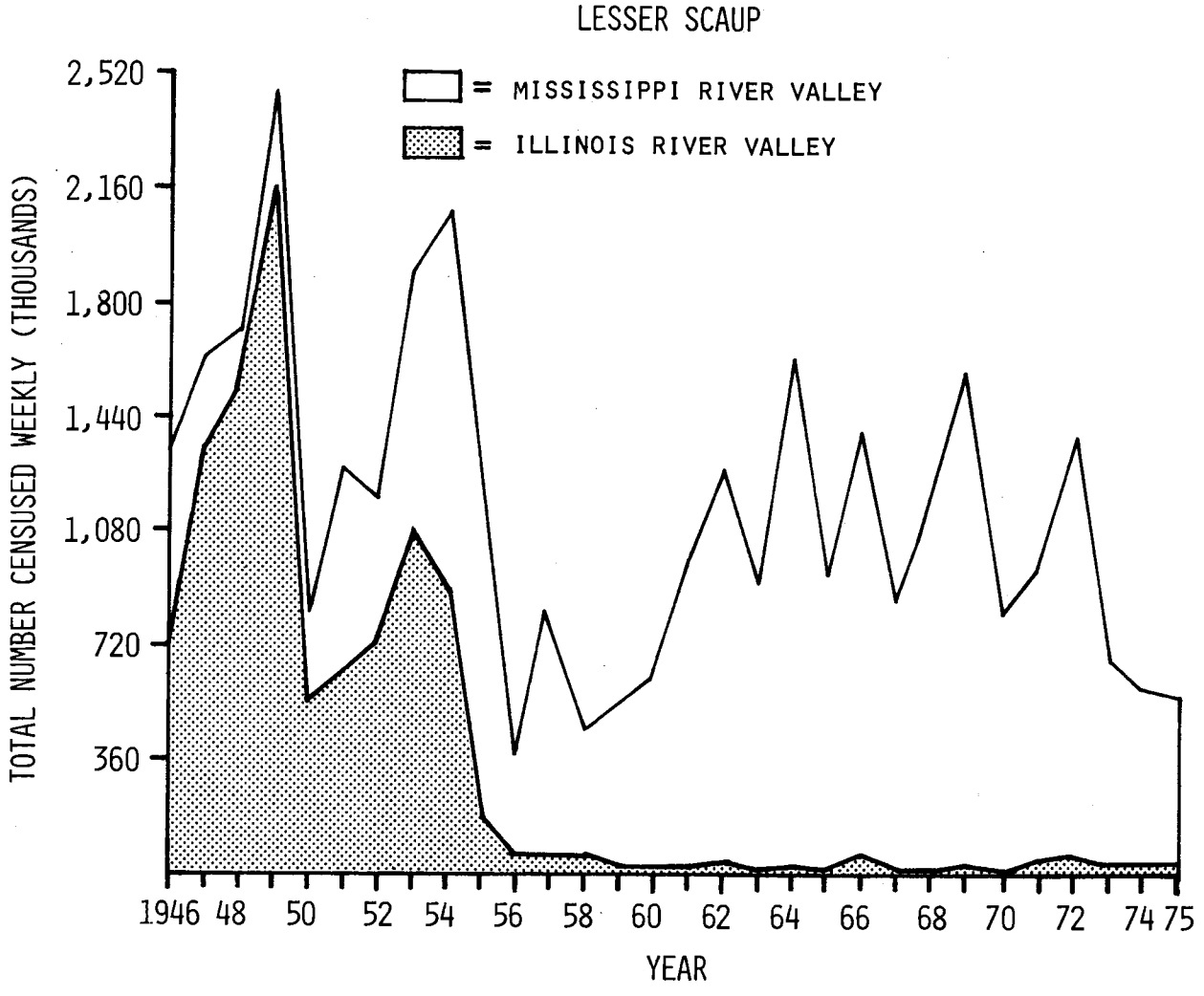
In the mid-1950's fingernail clams virtually disappeared from a 100-mile section of the Illinois River, a tributary of the Mississippi River, due to unknown causes (Paloumpis and Starrett, 1960: 406-435; Anderson, 1977: 3, 48-54). A survey of the bottom fauna of the Illinois River by Anderson in 1975 (Anderson, 1977: 5) revealed that fingernail clams were still absent from the middle reach of the River, where they had been abundant prior to the die-off in the 1950's. Fingernail clams can quickly repopulate an area when conditions are suitable. The clams can complete a life cycle in 33 days (Gale, 1969: v), and remnant "seed" populations are available in tributary streams and in an area of Peoria Lake where spring flow occurs (Anderson, 1977: 48-54). Some factor or factors in the Illinois River currently prevent the clams from recolonizing areas where they were formerly abundant. The unknown factor is probably the same one which caused the original die-off in the 1950's, although it is possible that the factor which eliminated the clams has been replaced by another factor which prevents recolonization.

As a result of the die-off of fingernail clams, the number of diving ducks, such as lesser scaups and canvasbacks, using the Illinois River during migration declined drastically (Mills, Starrett, and Bellrose, 1966: 18-20) and has never recovered since that time (Figure 2). Some of the ducks evidently shifted their migration route to the Mississippi River, where fingernail clams were still available (Mills et al., 1966: 18). Carp in the section of the Illinois River where the die-off of fingernail clams occurred are measurably thinner and smaller than downstream fish, probably because of poorer nutrition (Mills et al., 1966: 17). In the 1960's, fingernail clams formed 50.2 percent by volume of the food items taken by carp in sections of the river unaffected by the die-off, but only one clam was found in a carp from the affected section (Starrett, 1972: 151).

The disappearance of the fingernail clams in the Illinois River and the dramatic ecological repercussions of that disappearance illustrate the need for assessing water quality effects on lower organisms, in addition to fish. Apparently fingernail clams are more sensitive than fish to some factor in Illinois River water. If the effects of water quality factors on clams can be determined, it might be possible to make conditions in the Illinois River suitable for fingernail clams again and to prevent a similar ecological disaster from occurring in the Mississippi River. Restoration of fingernail clam populations in the Illinois River would dramatically increase fish production and diving duck utilization of the river.

In order to determine the effects of water quality on fingernail clams, a type of organism for which no standard testing methods exist, three methods were developed during this project: a rapid screening method, an acute bioassay method, and a chronic bioassay method. Shells of clams which had been

Figure 2. Use of the Illinois River by lesser scaup ducks plummeted following the die-off of fingernail clams in 1955 and has never recovered.



exposed to Illinois River water were also subjected to elemental analysis using X-ray microprobe techniques. The development of rapid screening methods is of particular importance, because of the length of time required to complete most acute and chronic bioassays and because of the accelerating rate of production of new chemicals which should be tested before being released to the environment.

# METHODS

## General Approach

It would take one lifetime, or perhaps several, to test all possible factors which might affect survival of fingernail clams. We took two approaches to reduce the burden of testing to a manageable size. Our first approach was to compare water quality in the Illinois River before and after the die-off of fingernail clams, and to compare recent water quality in the Illinois River with water quality in the Keokuk Pool, Mississippi River, where fingernail clams are still abundant. We used the annual summaries of data from the Water Quality Sampling Program of the Illinois Environmental Protection Agency (IEPA). The IEPA was established in 1970. Older data were obtained from the Illinois State Water Survey, which has had a water quality sampling station on the Illinois River at Peoria. We also used some additional water quality data obtained during biological surveys conducted by the Illinois Natural History Survey.

The second approach was to develop a method for rapidly assessing the effects of water quality factors on fingernail clams. The rapid method measures the average rate of beating of lateral cilia on excised clam gills. The beating rate of the lateral cilia is precisely regulated and coordinated. Changes in beating rate, or other changes, such as stoppage of the lateral cilia or a change from a metachronal to a synchronal pattern, occur rapidly (within 15 min to 1 h) in response to a variety to stimuli, and can be observed and measured within minutes by the method to be described in more detail below. Since the lateral cilia on the gills of the clam produce the water currents

which bring food and oxygenated water into the clam and carry wastes away, any impairment of ciliary function by a pollutant would be detrimental to the clam.

Once the rapid assessment technique had been used to determine which water quality factors had the greatest effect on fingernail clam gills, two of the water quality factors were selected for further testing using acute and chronic bioassay methods. It was possible that one level of a water quality factor would elicit a response from a gill preparation, but not affect the intact organism, where additional homeostatic mechanisms were operating. The acute and chronic bioassay methods were developed as part of this research, and we also investigated several alternative methods for determining when the clams had died.

## Collection of Fingernail Clams

Fingernail clams were collected from the Keokuk Pool of the Mississippi River using an 18-foot boat equipped with a crane and a Ponar grab sampler (Figure 3). Fingernail clams were separated from the mud by pressure-sieving the Ponar samples through a 30-mesh screen with a 12-volt battery-operated water pump. The clams were carried to the laboratory in 37-liter plastic coolers equipped with aerators and half-filled with Mississippi River water. Approximately 100 gallons of river water was pumped into a tank and brought back to the laboratory at Havana, Illinois.

## Rapid Screening Methods

Clams used in the rapid testing apparatus at Southern Illinois University were transported from Havana by truck or shipped via parcel post in plastic

Figure 3. Live fingernail clams, Musculium transversum, were obtained from the Keokuk Pool, Mississippi River, using a boat specially equipped with a crane and a Ponar grab sampler. Clams were separated from the mud by pressure sieving the Ponar samples through a 30-mesh screen mounted on the side of the boat.

bags or jars surrounded by styrofoam insulation to minimize temperature changes. The clams were delivered within 3 to 5 days of capture.

Clams used in the cilia monitoring apparatus were divided into small (1 to 5 mm shell length) and large (6 to 11 mm shell length) size classes and kept in separate tanks. They were acclimated at least one week in invertebrate physiological solution in two Instant Ocean aquaria (temperature 17 C and pH 7.8 to 8.2). Two of the chemicals tested, sodium and potassium, are components of the standard physiological solution used to maintain the clams and to bathe the isolated gills. In experiments where potassium or sodium was the test material, physiological solution was prepared without the test material. Reagent grade salts were then added to the solution to produce the desired concentration of the test material.

The effects of suspended particles on fingernail clam gills were determined by measuring the rate of transport of particles across isolated clam gills maintained in petri dishes. The clam gills were observed under a microscope, and the movement of particles across a known distance in the microscope field was timed. The effects of low dissolved oxygen concentrations in combination with suspensions of illite clay and silica flour were also determined.

The effects of the following 13 factors and factor combinations on the ciliary beating rate of fingernail clam gills were determined:

| | | |
|---|---|---|
| Temperature | Potassium Chloride | Ammonium Chloride |
| Dissolved Oxygen | Potassium Cyanide | Raw Illinois River Water and Sediment |
| Sodium Cyanide | Lead Nitrate | |
| Sodium Nitrate | Copper Sulfate | Low Dissolved Oxygen and Ammonium Chloride |
| Sodium Sulfate | Zinc Sulfate | |

The apparatus for monitoring ciliary activity of clam gills was specially constructed for this research (Figure 4). The apparatus consists of two coupled microscopes with an aluminum scanning stage which holds two petri dishes, each containing a gill preparation. Water from a constant-temperature bath circulates through coils embedded in the walls of the stage in order to control the temperature in the petri dishes.

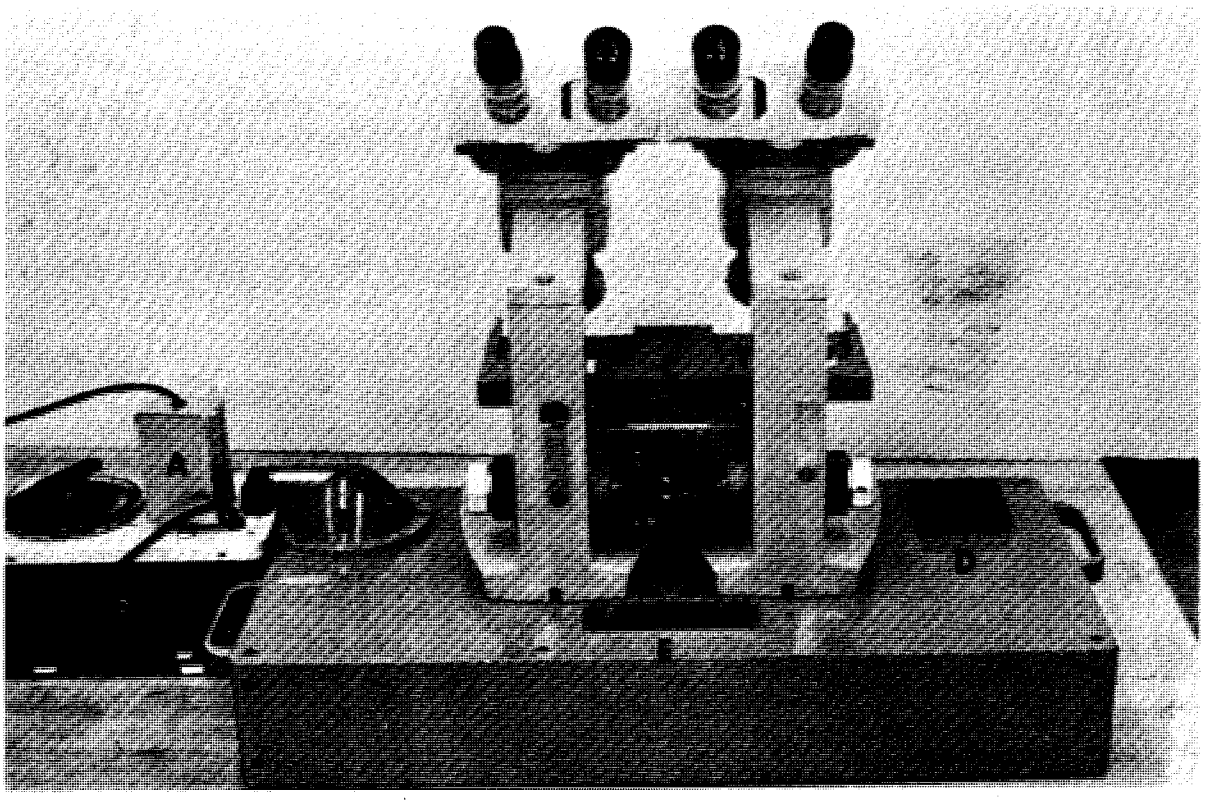


Figure 4. Apparatus for measuring the effects of water quality factors on the ciliary beating rate of clam gills.
A: Analog to digital converter on the stroboscopic light controller.
B: Stroboscopic light reflector leading to two light rods which transmit light to the stages of the microscope.
C: Motorized scanning stage with a hollow core for the circulation of water from a constant temperature bath.
D: Push-button control for the motorized stage. When a button is depressed, the stage will automatically return to a preselected point.
E: Digital display of ciliary beating rate.

Temperatures and dissolved oxygen concentrations in the petri dishes are monitored by thermistor meters and membrane electrodes. A continuous flow of standard physiological solution or solution to which test chemicals have been added can be maintained across the petri dishes by means of metering pumps.

Twelve sets of measurements are made at each observation time on each gill. The platform positions are initially adjusted so that comparable areas on two gills are examined at the same time, one gill appearing in the left microscope and one in the right. The scanning stage is controlled by a servo-mechanism capable of moving in an X and Y direction. Each point has its own X and Y coordinates and is independently isolated from the coordinates of the other eleven points. Once the twelve positions are locked into the stage-controlling mechanism at the beginning of the experiment, the observer can return to any of the twelve positions simply by pushing a button. The stage can be automatically returned to within ten μm of the original point.

A calibrated stroboscopic light serves as a substage light source for both microscopes. The light is divided and transmitted to the microscopes by means of a silver-coated Y-shaped Pyrex glass rod.

The rate of ciliary beating of lateral cilia (in beats per second) is measured by manually synchronizing the rate of flashing of the light with the rate of beating of the lateral cilia, which beat in a metachronal pattern. Synchronization is achieved when the metachronal wave appears to stand still. The beating rate is shown on a digital display.

Gills from a clam were isolated under a 30-power dissecting microscope and pinned to a rubber mat in a petri dish. In each experiment, 14 to 16 gill preparations were tested. Figure 5 is a diagram of generalized clam anatomy (the detailed anatomy of the fingernail clam differs somewhat from

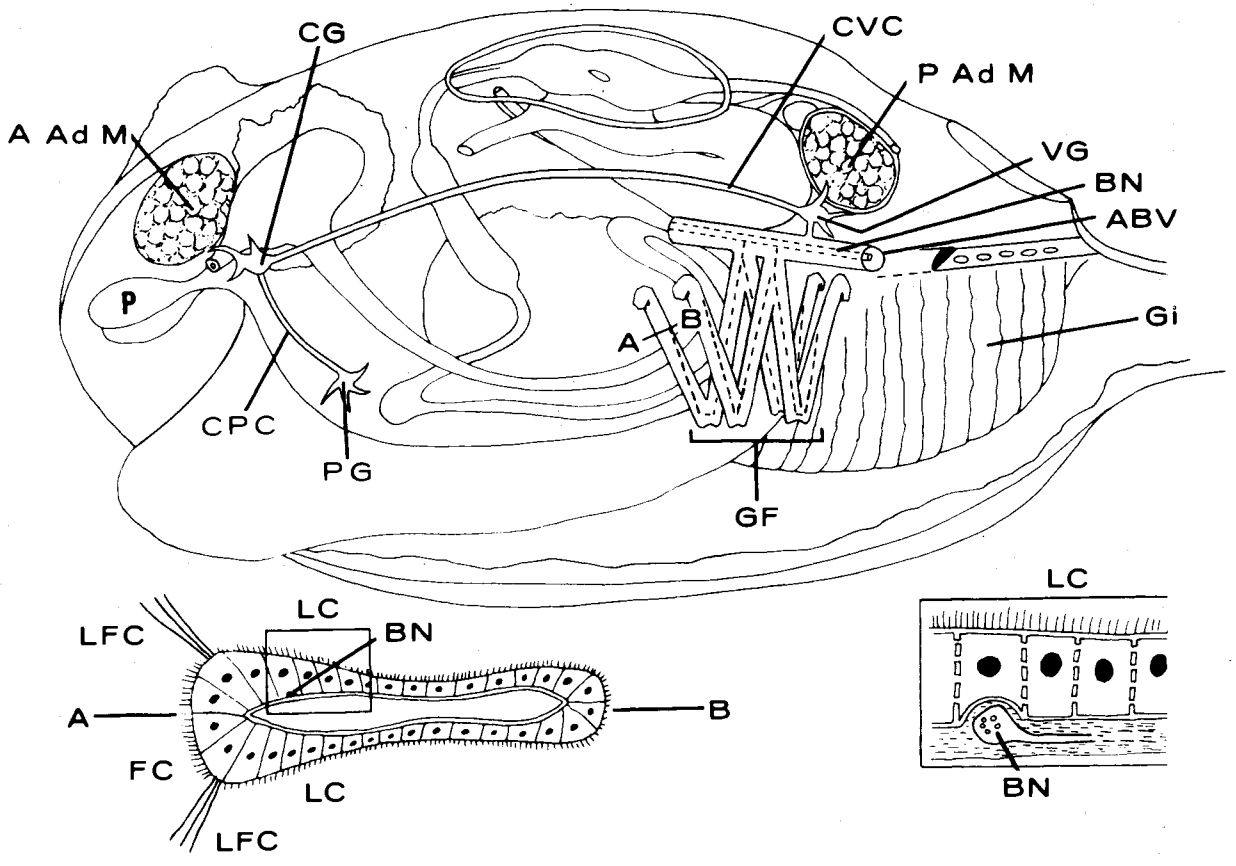



Figure 5. Generalized clam anatomy, showing the visceral ganglion (VG) and the gill (Gi), which are used in the cilia monitoring apparatus. (The detailed anatomy of the fingernail clam differs from that in the diagram.) The enlarged diagram of section A-B across a single gill filament (GF) shows the lateral cilia (LC). It is the beating rate of the lateral cilia which is monitored in the apparatus. The anterior adductor muscle (AAdM) and posterior adductor muscle (PAdM) close the shells. The paired palps (P) are oxygen sensors. The central nervous system consists of the pedal ganglion (PG), cerebral ganglion (CG), and visceral ganglion (VG). These ganglia are interconnected by the cerebral-pedal connective (CPC) and the cerebral-visceral connective (CVC). In cross section each gill appears in the form of a narrow "W". The inner and outer surfaces are made up of gill filaments (GF). Within the afferent branchial blood vessel (ABV), the branchial nerve (BN), which arises from the visceral ganglion, distributes its axons within the gill filament. The lateral cilia (LC) produce water currents, while the latero-frontal cilia (LFC) remove particles from the water.

that in the diagram) showing the visceral ganglion and the gill. Section A-B across a single gill filament shows the location of lateral cilia (LC), latero-frontal cilia (LFC), and frontal cilia (FC). The action of the cilia is partially controlled by the branchial nerve (BN) and visceral ganglion (VG). Figure 6 is a scanning electron micrograph of gill filaments from the blue mussel, Mytilus edulis, showing the gill filaments (GF), lateral cilia (LC), latero-frontal cilia (LFC), and frontal cilia (FC), which are similar to those in the fingernail clam. Responses of the lateral cilia to water quality factors were measured in this study.

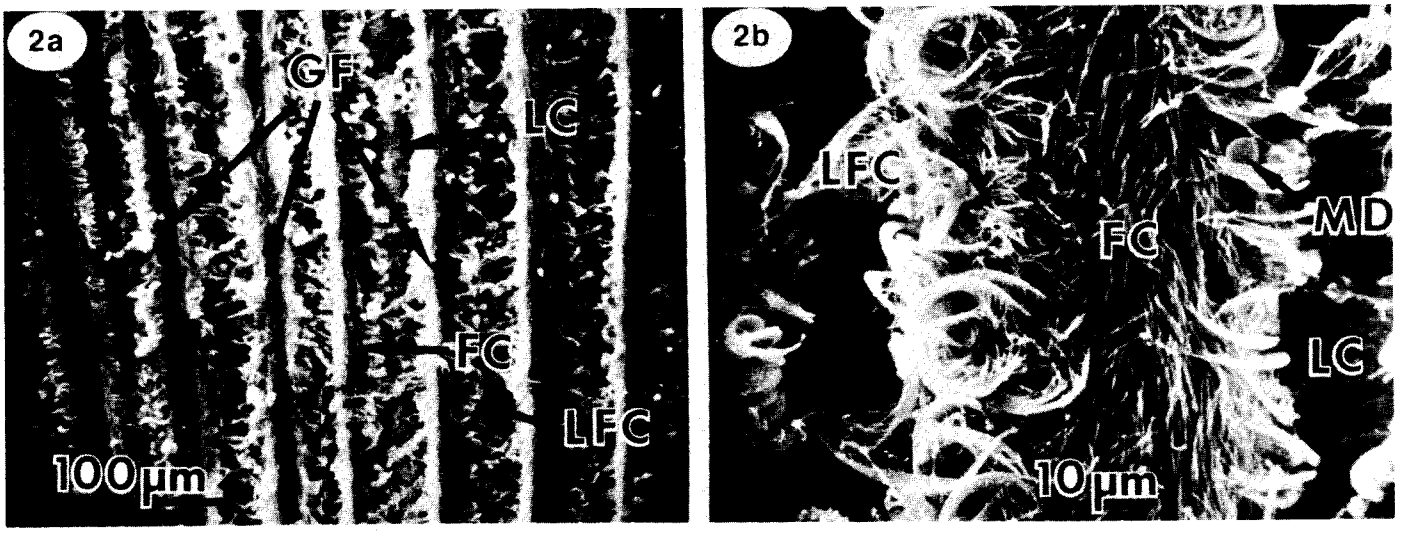



Figure 6. Scanning electron micrograph of gill filaments from the blue mussel, Mytilus edulis, showing the gill filaments (GF), lateral cilia (LC), latero-frontal cilia (LFC), and frontal cilia (FC), which are similar to those in the fingernail clam.

Acute Bioassay Methods

Clams used in the acute static bioassays were maintained in river water until they were acclimated to the test water. The clams were acclimated to the test waters as suggested by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).

Both adult clams (clams longer than 5 mm) and juvenile clams (5 mm and shorter) were used in bioassays. The bioassays will be identified consistently throughout the text by a J for tests conducted with juveniles and A for tests conducted with adults. A1 is the first test conducted with adults, A2 the second test, etc.

Most of the clams were exposed to a controlled light-dark cycle during acclimation and testing. During tests A2, A4, J2, and J3 the cycle was 14 hours of light and 10 hours of dark (Table 1). For the low-temperature test J4, the cycle was 10 hours of light and 14 hours of dark (Table 1) which approximates a natural winter cycle. The light sources were 40-watt cool-white fluorescent bulbs. Clams in acute tests A1 and J1 were not exposed to a controlled light-dark cycle, but to diffuse room light.

The dilution waters used in these experiments were from two different sources: unchlorinated well water from the Department of Conservation in Havana, Illinois, and reconstituted water (Marking, 1969). The chemical characteristics of the dilution waters were determined from samples collected at the beginning of each bioassay (Table 1). A logarithmic series of test solutions was prepared by diluting aliquots of known stock solution to 3 liters.

Heavy metal concentrations in the dilution water and test solutions were measured by either atomic absorption or flame photometry. Hardness, ammonia,

Table 1. Source and Chemical Characteristics of the Test Waters and Test Light-dark Cycles.

| Test | Water | Light-Dark Cycle, Hrs. Light | Dark | Potassium (mg/l) | Zinc (mg/l) | Copper (mg/l) | Lead (mg/l) | Total Hardness (mg/l as $CaCO_3$) | Total Ammonia (mg/l as $NH_3$-N) | Nitrite (mg/l as $NO_2$-N) | Nitrate (mg/l as $NO_3$-N) | Soluble Ortho-phosphate (mg/l as $PO_4$-P) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Adult acute static tests | | | | | | | | | | | | |
| A1 | DOC[a] | [c] | | 6.6 | 0.06 | 0.01 | 0.05 | 263 | 0.02 | 0.16 | 11.17 | 0.01 |
| A2 | RECON[b] | 14 | 10 | 2.6 | 0.01 | 0.01 | 0.05 | 243 | 0.22 | 0.01 | 0.01 | 0.26 |
| A3 | DOC | 14 | 10 | 6.6 | 0.06 | 0.01 | 0.05 | 263 | 0.02 | 0.16 | 11.17 | 0.01 |
| Juvenile acute static tests | | | | | | | | | | | | |
| J1 | DOC | [c] | | 6.6 | 0.06 | 0.01 | 0.05 | 263 | 0.02 | 0.16 | 11.17 | 0.01 |
| J2 | RECON | 14 | 10 | 2.6 | 0.01 | 0.01 | 0.05 | 243 | 0.22 | 0.01 | 0.01 | 0.26 |
| J3 | RECON | 14 | 10 | 2.0 | 0.01 | 0.01 | 0.03 | 314 | 0.05 | 0.01 | 0.09 | 0.01 |
| J4 | RECON | 10 | 14 | 2.8 | 0.03 | 0.01 | 0.05 | 234 | 0.19 | 0.01 | 0.01 | 0.22 |
| Chronic tests | | | | | | | | | | | | |
| K1 | DOC | [c] | | 13.0 | 0.08 | <0.01 | 0.01 | 243 | 0.09 | 0.13 | 12.72 | 0.03 |
| K2 | DOC | [c] | | 15.0 | 0.07 | <0.01 | 0.02 | 212 | 0.07 | 0.02 | 13.44 | 0.01 |
| $NH_3$2 | DOC | [c] | | 13.0 | 0.08 | <0.01 | 0.01 | 243 | 0.09 | 0.13 | 12.72 | 0.03 |
| $NH_3$3 | DOC | [c] | | 15.0 | 0.07 | <0.01 | 0.02 | 212 | 0.07 | 0.02 | 13.44 | 0.22 |

[a] DOC = unchlorinated well water from Department of Conservation, Havana, Illinois.
[b] RECON = reconstituted water (Marking, 1969).
[c] No controlled light-dark cycle.

nitrite, nitrate, and phosphate were measured by standard methods (American Public Health Association, 1976). To measure potassium, water samples were collected 3 times during each bioassay and analyzed by flame photometry, except in acute tests A1 and J1, where composite samples were used. In the ammonia tests, samples were collected for ammonia analysis from each test chamber 3 times weekly. Total ammonia concentrations were measured using a specific ion electrode and converted to undissociated ammonia using test pH and temperature and tables presented by Thurston et al. (1974).

Test chambers for the acute static bioassays consisted of 3.78-liter glass jars, which were immersed in a temperature-controlled water bath. Ten or 20 clams were contained in a petri dish (100 mm in diameter) covered with a plastic snap-on lid. A 50-mm diameter hole was cut in the center of the lid allowing circulation of water to the clams while preventing the clams from crawling out. One petri dish was placed in each test chamber. The petri dishes could be removed and examined with a dissecting microscope without greatly disturbing the clams. Clams were not fed during the test. Only clams that were actively siphoning or moving were selected for the tests.

Criterion for death was determined during the first adult and juvenile static bioassays, tests A1 and J1, respectively. Clams which gaped, or which did not withdraw their feet when prodded, were removed from the test concentrations and placed in clean water. They were checked 24 hours later for signs of recovery. Clams that recovered were not used in determining the concentrations lethal to 50 percent of the exposed clams (LC50).

Observation times varied slightly from one test to another, but in general the clams were checked for mortalities once every 24 hours for the first 96 hours, once every 48 hours from hours 96 to 240, once every 72 hours between

hours 240 and 456, and every 96 hours for the remainder of the test. Temperature and dissolved oxygen were measured at each mortality check and pH and total alkalinity were determined every other mortality check by standard methods (American Public Health Association, 1976).

For the acute bioassays the LC50's and their confidence limits were determined and statistically analyzed by the method of Litchfield and Wilcoxon (1949). To adjust for mortality in the control, Abbott's formula, given below, was used as suggested by the American Public Health Association (1976).

Abbott's Formula: $P = \frac{p'-c}{1-c}$, where:

P = corrected mortality

p' = observed mortality

c = control mortality

For example, with a control mortality of 20 percent and an observed test mortality of 60 percent, the corrected mortality would be 50 percent. Observed mortalities were adjusted in this fashion prior to applying the Litchfield and Wilcoxon (1949) method. A test mortality less than the control mortality was considered to be zero.

Acute toxicity curves were plotted according to Sprague (1973). LC50's were plotted with time to 50 percent mortality on the vertical axis and concentration on the horizontal axis. The resulting curve is the acute toxicity curve. The point on the concentration axis where the curve becomes asymptotic to the time axis defines the lethal threshold or the point where no more organisms are dying and the remaining organisms could presumably live indefinitely. A threshold was not considered valid unless the asymptotic portion of the curve was maintained for a period of time equivalent to at least 20 percent of the

time that was required to reach the asymptote, as suggested by Ruesink and Smith (1975). If the asymptote was approached but not completely attained, or if it was reached but did not meet the time criterion indicated above, it was considered a probable lethal threshold.

Toxicity curves were compared to determine relative toxicity of different factors and to determine how fast the factors induced effects.

## Chronic Bioassay Methods

Clams used in the chronic bioassays began acclimation to unchlorinated well water immediately upon arrival at a laboratory provided by the Illinois Department of Conservation at Havana. Acclimation procedures followed recommendations by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). The clams were exposed to natural daylight coming through north and west windows on the sides of the building.

The test chambers were 37.8-liter glass aquaria with outlets arranged so that the aquaria contained 23 liters of water (Figure 7). A modified proportional diluter (Mount and Brungs, 1967) was used to deliver a logarithmic series of test solutions to five aquaria and unchlorinated well water alone to the control aquaria (Figure 7). The effects of ammonia and potassium on fingernail clams were tested with this apparatus.

The effects of raw Illinois River water and sediment were tested by maintaining fingernail clams in cages in the Illinois River, and by exposing fingernail clams to both raw river water and river water diluted with well water in a laboratory located next to the Illinois River at Havana and provided by the Illinois Department of Conservation, Fisheries Division. River water containing suspended sediment was pumped into a reservoir in the laboratory,

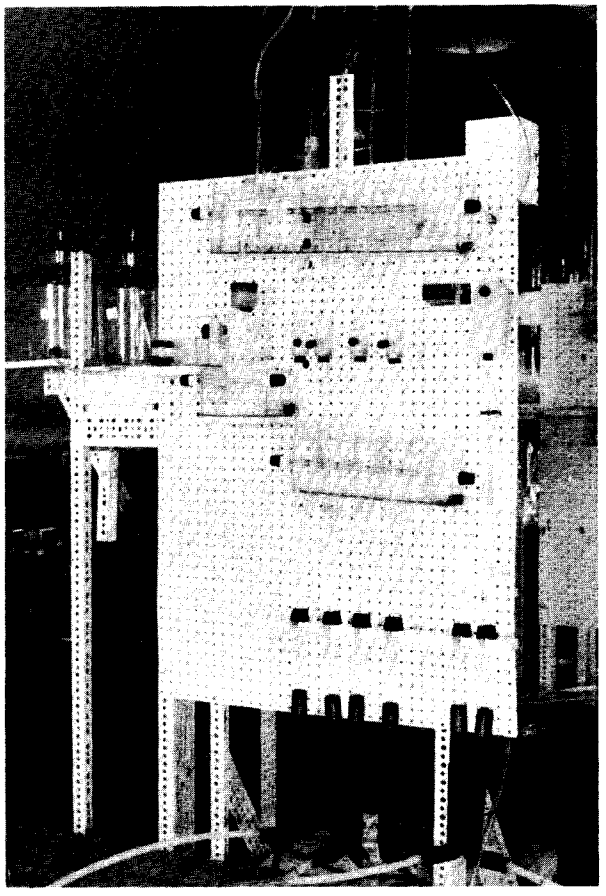


Figure 7. Toxicant diluters which delivered test solutions or well water to aquaria which served as test chambers for the chronic bioassays.

where a circulating pump kept the sediment in suspension and provided sediment-laden water to the diluter.

During preliminary testing of ammonia, we found that increasing concentrations of $NH_4Cl$ reduced the pH of the test solutions, thus necessitating the addition of NaOH in proportion to the concentration of $NH_4Cl$. This was accomplished by adding a second metering system on the diluter which once each cycle delivered a measured volume of a concentrated NaOH solution to the toxicant-mixing chamber. The NaOH metering system was calibrated to deliver 4 ml of a NaOH solution which varied in concentration between 0.025 M and

0.125 M depending on the pH of the test chambers. In addition we found that cleaning the entire testing system once a week aided in pH control.

A feeding system was also incorporated into the proportional diluter so as to deliver a measured amount of food once per diluter cycle. The food suspension was prepared as suggested by Biesinger and Christensen (1972). Preliminary testing showed the optimum feeding rate to be 0.80 ml of the food suspension per liter of test water.

In the chronic test 20 clams were used per petri dish with two petri dishes in each chamber. Survival and growth of the clams was checked once every two weeks. Growth was determined by measuring the maximum length of the shell to the nearest 0.1 mm with an ocular micrometer. Temperature and dissolved oxygen were measured 5 times a week, pH three times a week, and total alkalinity once a week except in two tests where pH was checked five times a week. Free carbon dioxide concentrations were calculated from the pH and alkalinity data using the indirect method of Rainwater and Thatcher (1960).

Data from the chronic bioassays were used to determine maximum acceptable toxicant concentrations (MATC) as suggested by the American Public Health Association (1976). MATC's were computed using both mortality and growth as responses. Replicate mortalities in each test chamber were subjected to analysis of variance, ANOVA (Steel and Torrie, 1960). When treatment effects were indicated by ANOVA, the means of these effects were subjected to the Newman-Keul's multiple-range test (Steel and Torrie, 1960). Pooled clam lengths from each test chamber were also subjected to analysis of variance and the Newman-Keul's multiple-range test. Data collected from test chambers showing mortalities significantly higher than the controls were not used in the

analysis of clam lengths. All differences were considered statistically significant at a probability of $p = 0.05$.

Elemental Analysis of Shells

Dr. Judith Murphy, Director of the Center for Electron Microscopy at Southern Illinois University -- Carbondale, determined the elemental composition of shells from fingernail clams which had been chronically exposed to raw Illinois River water or to ammonia. She used X-ray microprobe techniques to determine relative amounts of specific elements in specific regions of the shells, starting from the hinge area and proceeding outwards to the shell margin. Since the clam grows by adding to the shell at the margin, chronological changes in the composition of the shell were determined.

# RESULTS AND DISCUSSION

## Water Quality in the Illinois River in the 1950's

When the fingernail clams died out in portions of the Illinois River in the mid-1950's, relatively few analyses of toxic substances in Illinois River water were being made and the analytical methods were not sensitive enough to detect concentrations of parts per million or parts per billion which can affect aquatic organisms. Some water quality factors which are generally considered non-toxic were measured by the Illinois State Water Survey at Peoria during the 1950's. These data show that sodium and chloride concentrations in the Illinois River have increased since 1950, partly due to the increasing use of salt to melt ice on streets and highways.

Dr. Ronald Flemal, Associate Professor of Geology, Northern Illinois University, speculated in a letter dated 5 May, 1976 that the potassium concentrations in the Illinois River may have increased during the same period also:

> The Water Survey . . . [has] two stations on the main stem (Peoria and Meredosia), and potassium analyses only at Peoria for the period 1966-71. . . .
>
> In the absence of actual potassium data, I am not sure if we can say much positive about past trends in potassium concentrations; the best we can do is speculate. . . .
>
> The chloride data display an obvious upward trend, with a particularly significant upward step in 1962-63. Chloride enters the environment in many ways . . . . One of the more important of these is as potassium chloride fertilizer, and the 62-63 period is about when potassium chloride fertilizer became widely used. One might expect therefore that potassium concentrations (at least during the fertilizing season) turned upward in mirror to the chloride concentrations.
>
> A somewhat similar case can be made from the "sodium" data. I give the data in quotation marks because previous to 1966 the Water Survey determined sodium by calculation. This means that the reported values of sodium are actually sodium and potassium plus assorted lesser cations. Two observations then make the case: (1) the sodium data closely

> correspond to the chloride data, and (2) the differential between the chloride and sodium lines decreases in 1966 when the potassium is actually split out from the "sodium." These two imply that the potassium is a significant part of the "sodium" previous to 1966 and that as the "sodium" curve rises, so also ought a potassium curve have risen if it could have been calculated.
>
> These are admittedly not the strongest of arguments for an historical change in potassium concentrations, but they are the best I can make at the moment and without further investigation.

Potassium is nontoxic to fish, but Imlay (1973: 97) demonstrated that potassium concentrations of 11 mg/l killed several species of mussels in 1-2 months.

Mills et al. (1966: 9) provide a table, compiled from various sources, of minimum dissolved oxygen levels near the surface in the channel of the Illinois River from 1911 through 1965. In 1950, the average dissolved oxygen concentration was 4.0 (range 2.9-5.3). There is a gap in the data until 1964, when the average oxygen concentration in the same reach was 2.7 mg/l (range 2.0-5.3). Conditions were even worse in 1965, when the average was 2.3 mg/l and the range 1.0-5.6. The minimum dissolved oxygen levels in the Illinois River evidently declined after 1950.

To summarize: (1) There are few historical data available on concentrations of toxic substances in the Illinois River in the 1950's, (2) available data show that concentrations of sodium, chloride, and possibly potassium increased in the river in the 1950's, and (3) dissolved oxygen levels in the river may have decreased in the 1950's.

## Comparison of Water Quality in the Mississippi and Illinois Rivers in 1975

Since fingernail clams have never recolonized the Illinois River since the die-off in the 1950's, comparison of present water quality in the Illinois River with water quality in the Mississippi, where the clams are still abundant, might indicate which water quality factors are affecting the clam. The

Illinois Environmental Protection Agency (IEPA) currently analyzes water samples from the major rivers of the state for twenty-eight water quality factors.

Tables 2, 3, and 4 show mean, median, and maximum values of sixteen water quality factors which occurred at higher levels in 1975 at four sampling stations on the Illinois River than at a sampling station on the Keokuk Pool, Mississippi River. The four stations are in a reach of the Illinois River where fingernail clams died out in the 1950's. One other factor, dissolved oxygen, was generally lower in the Illinois River than in the Mississippi River.

The following four factors regularly occurred at substantially higher concentrations in the Illinois River than in the Mississippi River and are known to be toxic to fish: ammonia, lead, fluoride, and methylene blue active substances. Nitrite is less toxic to fish than ammonia, but more toxic than nitrate. Unfortunately, nitrite and nitrate are reported as a combined value by the Illinois Environmental Protection Agency. Counts of fecal coliform bacteria were much higher in the Illinois than in the Mississippi, but clams feed on bacteria, so it is not likely that increased bacterial populations adversely affected fingernail clams. The maximum water temperature in midsummer of 1975 in the Keokuk Pool, Mississippi River, differed from the maximum in the Illinois River by only $1^{o}$ F (Table 4). Although the median and mean water temperatures were higher in the Illinois than in the Mississippi (Tables 2 and 3), the range of the fingernail clam Musculium transversum extends into southern parts of the United States, where the mean water temperature equals that in the Illinois River. Total dissolved solids, chloride, phosphorus, and sulfate were higher in the Illinois River than in the Mississippi, but these factors are not considered toxic to fish.

Table 2.[a] Mean Values of Water Quality Factors Which Occurred at Higher Levels[b] in the Middle Section of the Illinois River, Where Fingernail Clams Died Out, Than in the Keokuk Pool, Mississippi River, Where Fingernail Clams Were Still Abundant Through 1975.

| Water Quality Factors | Location | | | | |
|---|---|---|---|---|---|
| | Mississippi River[c] | | Illinois River[d] | | |
| | Rt. 9 Bridge Ft. Madison, IA Mile 383.9 | Rt. 150 Bridge Peoria, IL Mile 165.8 | Lock and Dam Creve Coeur, IL Mile 157.7 | Rt. 9 Bridge Pekin, IL Mile 152.9 | Rt. 97 Bridge Havana, IL Mile 119.5 |
| Water Temperature, $^{o}F$ | 55 | 57 | 57 | 61 | 56 |
| Dissolved Oxygen, mg/l | 10.6 | 9.8 | 9.0 | 8.9 | 8.7 |
| Total Phosphorus, mg/l | 0.286 | 0.483 | 0.539 | 0.542 | 0.437 |
| Fecal Coliform, #/.1 l | 268 | | 1586 | 1006 | 761 |
| $NH_3$-N, mg/l | 0.22 | 0.55 | 0.54 | 0.61 | 0.49 |
| $NO_3$-N + $NO_2$-N, mg/l | 1.1 | 4.6 | 4.7 | 4.8 | 4.0 |
| Total Arsenic, mg/l | 0.0 | | 0.001 | | |
| Total Lead, mg/l | 0.0 | 0.02 | 0.01 | | 0.02 |
| Total Dissolved Solids, mg/l | 219 | 390 | | | 440 |
| Fluoride, mg/l | 0.1 | 0.4 | 0.6 | 0.6 | 0.4 |
| Chloride, mg/l | 11 | 45 | 56 | 57 | 29 |
| Sulfate, mg/l | 26 | 81 | 104 | 103 | 104 |
| Total Boron, mg/l | 0.1 | | 0.2 | 0.3 | |
| Methylene Blue Active Substances, mg/l | 0.20 | 0.76 | | 0.50 | 0.70 |

[a]Data were obtained from the Illinois Environmental Protection Agency, 1975 Summary of Data, Water Quality Network, Volumes 2 and 4. Blanks indicate that the mean value in the Illinois River was less than or equal to the value in the Keokuk Pool.

[b]Except for dissolved oxygen, where levels were lower in the Illinois River.

[c]This station is in the middle section of Keokuk Pool. Mile refers to miles above the confluence of the Ohio and Mississippi Rivers near Cairo, Illinois.

[d]Mile refers to miles above the confluence of the Illinois and Mississippi Rivers near Grafton, Illinois.

Table 3.[a] Median Values of Water Quality Factors Which Occurred at Higher Levels[b] in the Middle Section of the Illinois River, Where Fingernail Clams Died Out, Than in the Keokuk Pool, Mississippi River, Where Fingernail Clams Were Still Abundant Through 1975.

| Water Quality Factors | Location | | | | |
|---|---|---|---|---|---|
| | Mississippi River[c] | Illinois River[d] | | | |
| | Rt. 9 Bridge Ft. Madison, IA Mile 383.9 | Rt. 150 Bridge Peoria, IL Mile 165.8 | Lock and Dam Creve Coeur, IL Mile 157.7 | Rt. 9 Bridge Pekin, IL Mile 152.9 | Rt. 97 Bridge Havana, IL Mile 119.5 |
| Water Temperature, $^{o}F$ | 57 | 61 | 63 | 68 | 58 |
| Dissolved Oxygen, mg/l | 10.6 | 9.0 | 8.6 | 8.3 | 8.4 |
| Total Phosphorus, mg/l | 0.235 | 0.43 | 0.51 | 0.52 | 0.44 |
| Fecal Coliform, #/.1 l | 225 | | 1850 | 800 | 610 |
| $NH_3$-N, mg/l | 0.09 | 0.31 | 0.26 | 0.52 | 0.36 |
| $NO_3$-N + $NO_2$-N, mg/l | 1.4 | 4.8 | 5.2 | 4.8 | 4.4 |
| Total Copper, mg/l | 0.0 | | | | 0.01 |
| Total Lead, mg/l | 0.0 | 0.01 | 0.01 | | 0.02 |
| Total Dissolved Solids, mg/l | 230 | 390 | | | 440 |
| Fluoride, mg/l | 0.2 | 0.4 | 0.8 | 0.6 | 0.4 |
| Chloride, mg/l | 12 | 43 | 59 | 57 | 28 |
| Sulfate, mg/l | 24 | 84 | 112 | 103 | 94 |
| Total Boron, mg/l | 0.1 | 0.2 | 0.2 | 0.3 | 0.2 |
| Total Iron, mg/l | 2.1 | | | 2.2 | 2.3 |
| Methylene Blue Active Substances, mg/l | 0.20 | 0.90 | | 0.50 | 0.70 |

[a] Data were obtained from the Illinois Environmental Protection Agency, 1975 Summary of Data, Water Quality Network, Volumes 2 and 4. Blanks indicate that the median value in the Illinois River was less than or equal to the value in the Keokuk Pool.

[b] Except for dissolved oxygen, where levels were lower in the Illinois River.

[c] This station is in the middle section of Keokuk Pool. Mile refers to miles above the confluence of the Ohio and Mississippi Rivers near Cairo, Illinois.

[d] Mile refers to miles above the confluence of the Illinois and Mississippi Rivers near Grafton, Illinois.

Table 4.[a] Maximum Values of Water Quality Factors Which Occurred at Higher Levels[b] in the Middle Section of the Illinois River, Where Fingernail Clams Died Out, Than in the Keokuk Pool, Mississippi River, Where Fingernail Clams Were Still Abundant Through 1975.

| Water Quality Factors | Location | | | | |
|---|---|---|---|---|---|
| | Mississippi River[c] | Illinois River[d] | | | |
| | Rt. 9 Bridge Ft. Madison, IA Mile 383.9 | Rt. 150 Bridge Peoria, IL Mile 165.8 | Lock and Dam Creve Coeur, IL Mile 157.7 | Rt. 9 Bridge Pekin, IL Mile 152.9 | Rt. 97 Bridge Havana, IL Mile 119.5 |
| Water Temperature, $^{o}F$ | 81 | | | | 82 |
| Dissolved Oxygen, mg/l (minimum) | 7.0 | 4.7 | 5.0 | 4.9 | 4.9 |
| Total Phosphorus, mg/l | 0.84 | 0.96 | 0.87 | 0.89 | |
| Fecal Coliform, #/.1 l | 1300 | | 5300 | 11,000 | 13,000 |
| $NH_3$-N, mg/l | 1.3 | 2.6 | 1.9 | 2.2 | |
| $NO_3$-N + $NO_2$-N, mg/l | 2.2 | 7.6 | 6.5 | 7.6 | 6.0 |
| Total Arsenic, mg/l | 0.001 | | 0.003 | | |
| Total Lead, mg/l | 0.0 | 0.06 | 0.02 | 0.01 | 0.04 |
| Total Dissolved Solids, mg/l | 260 | 440 | | | 440 |
| Fluoride, mg/l | 0.2 | 0.8 | 0.8 | 0.8 | 0.5 |
| Chloride, mg/l | 12 | 63 | 74 | 74 | 39 |
| Sulfate, mg/l | 34 | 101 | 115 | 117 | 155 |
| Total Boron, mg/l | 0.3 | 0.5 | 0.4 | 0.4 | |
| Total Mercury, mcg/l | 0.1 | 0.5 | | | |
| Methylene Blue Active Substances, mg/l | 0.2 | 0.9 | | 0.5 | 0.7 |

[a] Data were obtained from the Illinois Environmental Protection Agency, 1975 Summary of Data, Water Quality Network, Volumes 2 and 4. Blanks indicate that the maximum value in the Illinois River was less than or equal to the value in the Keokuk Pool.

[b] Except for dissolved oxygen, where levels were lower in the Illinois River.

[c] This station is in the middle section of Keokuk Pool. Mile refers to miles above the confluence of the Ohio and Mississippi Rivers near Cairo, Illinois.

[d] Mile refers to miles above the confluence of the Illinois and Mississippi Rivers near Grafton, Illinois.

It is difficult, if not impossible, to relate the die-off of fingernail clams in the Illinois River to water quality factors, because the water quality requirements of fingernail clams are not known. The next two subsections report the results of our efforts: (1) to develop rapid methods for assessing the effects of water quality on fingernail clams and (2) to develop standard acute and chronic bioassay procedures, including a reliable indicator of death, using clams as test organisms.

## Reliability of the Rapid Screening Methods

At 21° C with an oxygen concentration of 6.5 mg/l and a pH of 7.6, the beating of the lateral cilia on the excised gill preparation was rapid, well coordinated, and fairly constant after two hours of equilibration. The gill preparation seemed to be fairly robust, because normal ciliary activity was maintained for at least eight days. The standard deviations of the mean ciliary beating rates are reported in the tables or plotted on the graphs which follow in the rest of the results section. Only the standard deviations in the positive direction are plotted (as one half of a bracket), to avoid cluttering the figures. In general the standard deviations were quite small and uniform. Even small changes in the levels of various water quality factors produced marked changes in the average ciliary beating rates, with little or no overlap of the standard deviations.

The particle transport rate across the gills proved to be similarly reliable and sensitive.

The sensitivity of the rapid method is compared to the sensitivity of the chronic bioassay method in the results subsections on potassium and ammonia.

## The Gaping Response as an Indicator of Death

Death was best indicated by the gaping response. Gape was due to relaxation of posterior and anterior adductor msucles, after which the elastic external ligament forced the shells open. The clam did not respond when the body was prodded through the open shell and was incapable of keeping the shell closed when the valves were forced together. Clams failed to recover after exhibiting this response.

The death criterion was verified in tests A2 and J2. Clams exhibiting the gaping response were removed and placed in clean water. In both of these tests, 100 percent of the clams failed to recover after exhibiting this response.

Some adults exhibited a partial gape which was not a valid death indicator. A partial gape was characterized by a valve separation of 1 mm or less in conjunction with a lack of response to prodding. Clams recovered from this condition if placed in clean water. Juvenile clams never exhibited a partial gape.

Other responses that the clams exhibited that were not death indicators were immobilization, cessation of heart beat (visible through the transparent shell), contraction of body toward the umbo region, and lack of response to prodding of the extended foot or siphon upon which the valves were tightly closed.

## Acute and Chronic Bioassay Methods with Juvenile and Adult Fingernail Clams

Two types of bioassay methods were used in this research: the acute static bioassay in which the clams were not fed, and the chronic bioassay in which the clams were fed and effects of the toxicant on growth were monitored.

The chronic bioassay was the preferred method of testing M. transversum for several reasons. The slow response of the clam is a primary consideration. Sprague (1973) stated that acute bioassays should not be terminated until the toxicity curve becomes asymptotic to the vertical axis and thus indicates a lethal threshold. Only adult test A2 (Figure 22 ) showed a valid lethal threshold after 240 hours (10 days) of exposure. Acute static tests J3 and J4 (Tables 16 & 18) were extended beyond 600 hours (25 days) without demonstrating lethal thresholds. In general, the main advantage of using acute bioassay techniques is reduced testing time, e.g. in most fish bioassays a lethal threshold can be reached in 24 to 48 hours. This advantage was not evident in the acute tests with Musculium transversum. On the other hand, the species seems to be ideally suited for chronic bioassay. A properly conducted chronic bioassay should expose the organism to the toxicant for an entire life cycle. M. transversum, under optimum conditions, can complete an entire life cycle in 33 days (Gale, 1969).

There were certain deficiencies in the chronic testing system. These are related mainly to improvements that are needed in the culture method. Gale (1969) stated that 5 mm was the minimum length of clam in which embryos normally occur. The best growth that was achieved in the chronic bioassays was in potassium test K2 where clams in one test chamber grew an average of 1.4 mm in 28 days (Figure 26 ). This growth rate needs to be nearly doubled if reproduction is to be obtained within the optimum 33 days. The reasons for the reduced growth rate are unknown and experiments are currently being conducted to make improvements in the culture system.

Toxicity curves of adults and juvenile clams under similar test conditions were compared (Figures 21 and 22 ) and were significantly different ($p=0.05$). Adults responded 5 to 1.6 times faster than the juveniles to

potassium. A lethal threshold was obtained at least 150 hours faster in the adult test than in the juvenile test (Figure 22 ).

The most important reason for using M. transversum as a bioassay organism relates to its paramount ecological importance, as described in the Introduction and Background.

The responses of clams to light, raw Illinois River water, cyanide, low dissolved oxygen concentrations, and six of the sixteen factors which occurred at higher levels in the Illinois River than in the Mississippi River are described in the subsections which follow.

## Response of Clams to Light and Darkness

Volumes have been written on the role of light and darkness in controlling physiological processes in both plants and animals (for example, Beck, 1963). It was important to determine the effects of light and darkness on fingernail clams. The lighting at the laboratory could then be controlled to avoid confounding the effects of lighting with the effects of the water quality factors.

Figure 8 shows that the ciliary beating rates of gills from two Sphaeriacean clams (Musculium transversum and Corbicula manilensis) and the unrelated intertidal mussel, Mytilus edulis, were inhibited by light from a fluorescent desk lamp containing two Sylvania F-15/T8 CW tubes positioned about 0.5 m from the gill preparations. Noticeable inhibition occurred after 1 to 2½ hours of exposure to light and maximum inhibition occurred after 2 to 6 hours' exposure. When the gill preparations were returned to darkness, the ciliary beating rates returned to normal after 2 to 4 hours, with the exception of large Musculium transversum, whose ciliary beating rates had not recovered to normal by the time the experiment was ended after 4 hours exposure to darkness. The

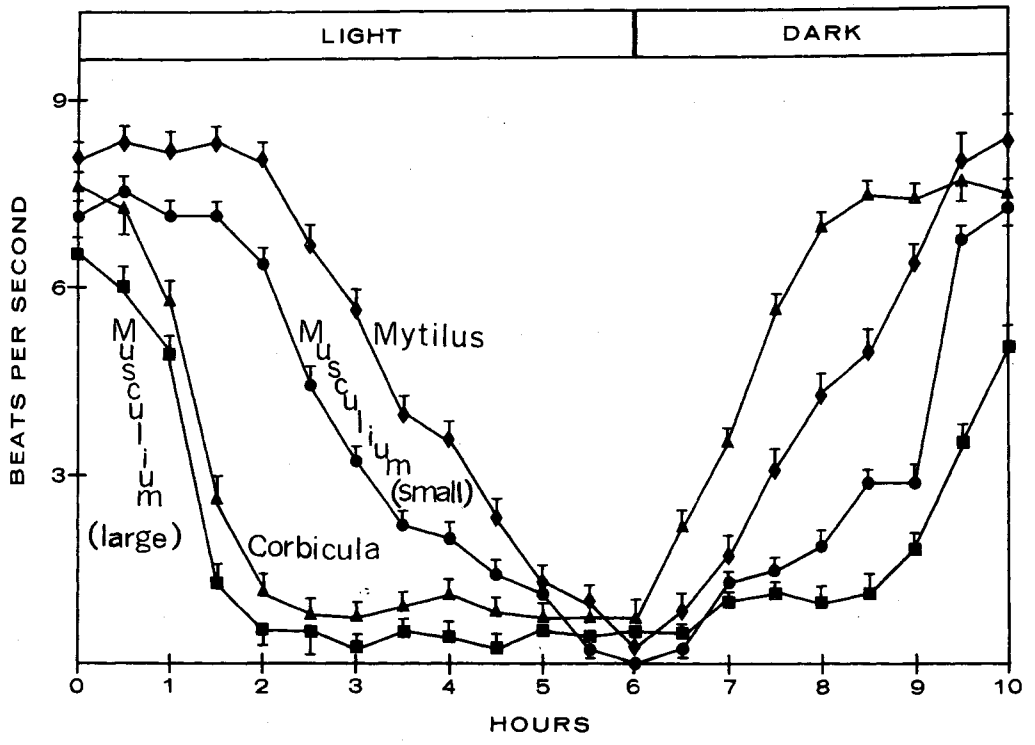



Figure 8. Light inhibits the beating of lateral cilia on the gills of two Sphaeriacean clams (Musculium transversum and Corbicula manilensis) and the unrelated intertidal mussel, Mytilus edulis. Beating rates recovered when the light was turned off.

response of small fingernail clams to light was more similar to that of the intertidal mussel, than to the large fingernail clams.

Musculium transversum lives in almost perpetual darkness in or on the bottom of the turbid Mississippi River. However, there are a few occasions when light does reach the bottom, such as in mid-winter, under ice, a time when the clam is normally dormant in Keokuk Pool, Mississippi River. Perhaps the inhibition of the cilia by light is a protective response, should the clam be exposed to falling water levels or to the air. The shell of the fingernail clam is certainly translucent and practically transparent. It would be interesting to determine whether the clam feeds more actively at night when it

lives in very shallow, or clear water. Perhaps the increasing exposure to daylight, which would result from decreasing water levels over shallow areas, would stimulate the clams to burrow into the mud and cease normal activity (including ciliary activity) thus protecting them from desiccation.

All subsequent experiments with water quality factors were conducted in a photographic darkroom, because the maximum ciliary beating rate was obtained in darkness. The only illumination was provided by a photographic safe light with a Kodak 1A filter. The safe light did not influence the ciliary beating rate. Stroboscopic measurement of the ciliary beating rate took only a few seconds, and was not long enough to cause inhibition of the cilia. Intact clams were exposed to natural light from windows or timer-controlled lights during the chronic and acute bioassays, except for acute tests A1 and J1 where they were exposed to diffuse, low-intensity room light.

## Response of Clams to Temperature

Gill preparations from the intertidal mussel, Mytilus edulis, maintained their ciliary beating rates over a broader temperature range than gill preparations from fingernail clams or Asiatic clams (Figure 9). It is not surprising that the intertidal mussel shows such a broad temperature range, for it must withstand daily exposure to the hot sun when the tide is out, followed by sudden immersion in cold sea water when the water returns. Small fingernail clams have a broader temperature range than large ones, and the Asiatic clam appears to have the narrowest temperature tolerance, at least as measured by the ciliary beating response.

We have found that fingernail clams begin to grow rapidly when water temperatures in the Keokuk Pool, Mississippi River rise above 11-13 C. Figure 9 shows that the ciliary beating rate of the fingernail clam substantially

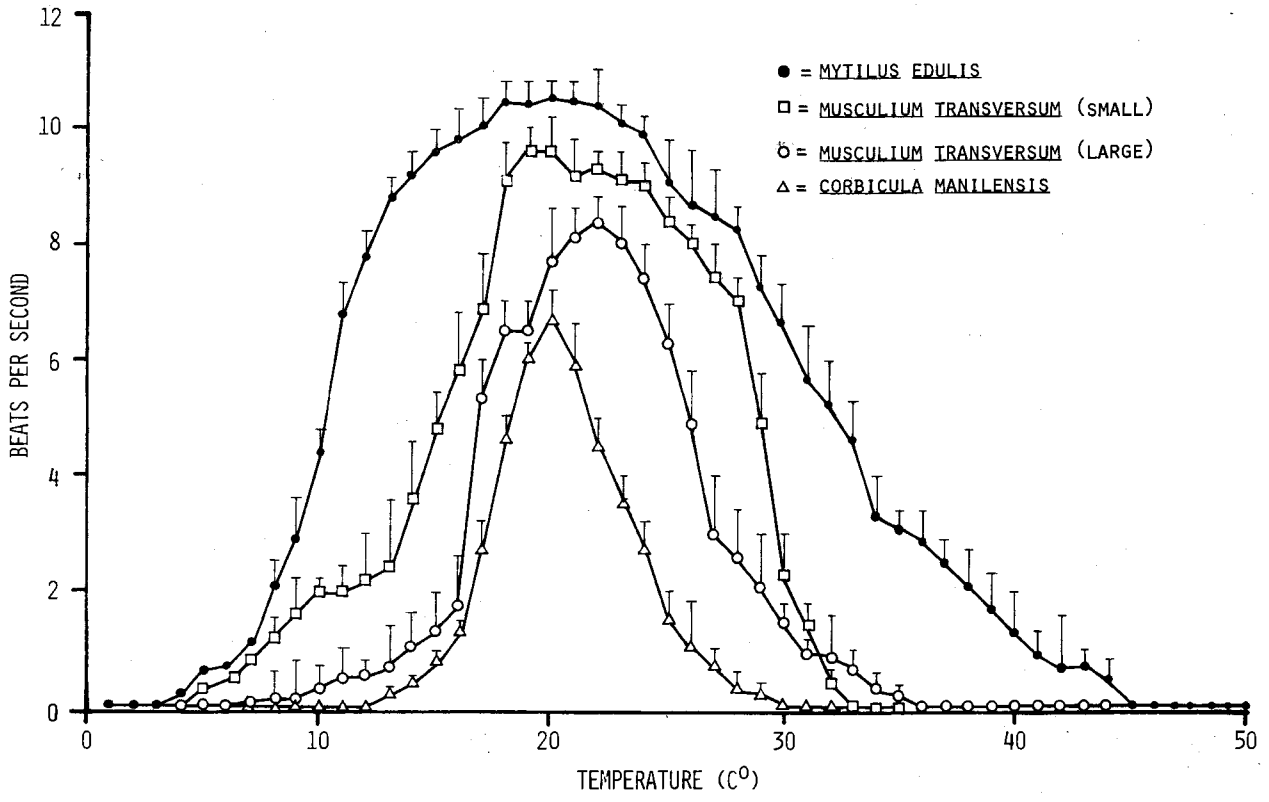



Figure 9. Ciliary beating response of gills from large and small fingernail clams (Musculium transversum), the Asiatic clam (Corbicula manilensis), and the blue mussel (Mytilus edulis) to temperature.

increased above 14-15°C, so the field results appear to corroborate the laboratory findings. The laboratory results also suggest that the ciliary function of fingernail clams would be drastically reduced if water temperatures increased above 30°C.

It is surprising that the gills from Asiatic clams showed a maximum beating response at 20°C, and a beating rate of practically zero at 30°C, although Asiatic clams are known to inhabit thermal effluents which exceed 30°C. (Personal communication, February 10, 1978, Mr. Herbert Dreier, Aquatic Biologist, Illinois Natural History Survey.) Mattice and Dye (1976) report that the upper temperature tolerance is 34°C, for Asiatic clams

acclimated to 30°C. However, all gill preparations used in our experiments came from clams which had been acclimated to water temperatures of 17°C, and it is likely that the optimum temperature for the ciliary beating response would be shifted toward higher temperatures, if the clams were acclimated to higher temperatures. Additional experiments would have to be performed to determine the acclimation range of these three species of clam, and the relationship between the ciliary beating response and acclimation temperature. Figures 10 and 11 show that the ciliary beating rate of gills from both large and small fingernail clams is proportional to the water temperature. The small fingernail clams showed a greater response to increased water temperature than to large clams. When the temperature was increased from 18° to 21°C, the ciliary beating rate of small clams increased from 9 to 20 beats per second, whereas the ciliary beating rate of gills from large clams increased from 8 to 11 beats per second for just a few seconds. The effects of potassium cyanide and sodium cyanide are discussed in another subsection.

Response of Clams to Dissolved Oxygen

Figure 12 shows that the higher the concentration of dissolved oxygen in the water perfusing the gill preparation, the greater the ciliary beating rate. The ciliary beating rate of gill preparations from large clams rapidly declined to zero when the dissolved oxygen concentration was reduced to 2 parts per million. Gill preparations from small clams showed a similar response, although the results are not plotted in Figure 12 for the sake of clarity. It is unlikely that dissolved oxygen concentrations of 2 parts per million or lower would immediately kill fingernail clams in nature. Most, or perhaps all clams can switch from aerobic to anaerobic metabolism when their shells are closed. We did not conduct any acute or chronic bioassays to

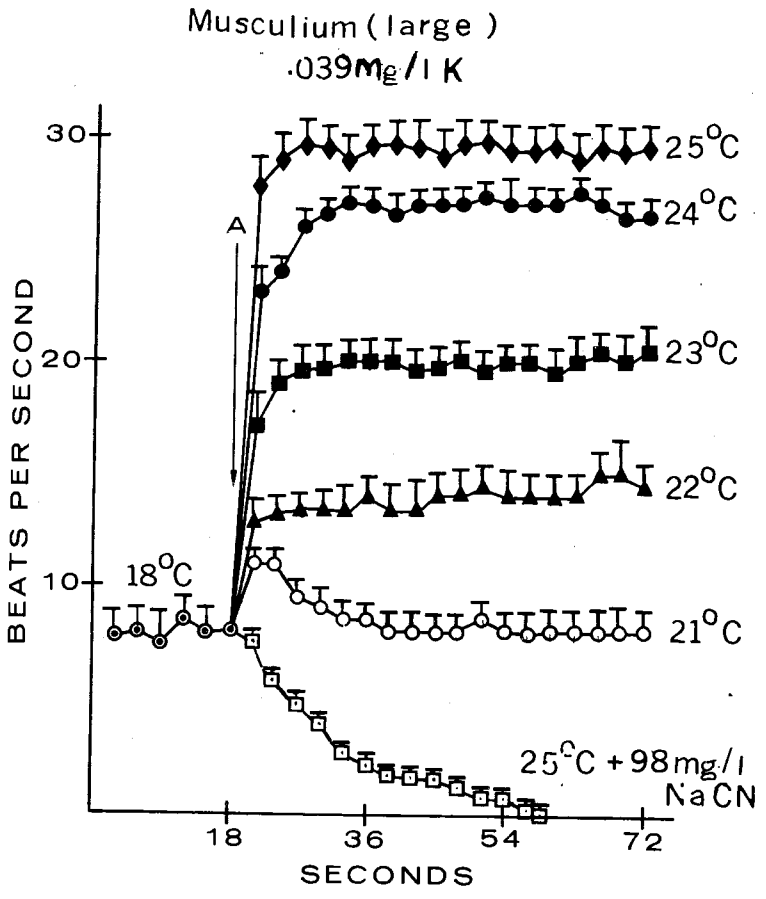



Figure 10. Beating rate of cilia on gills from large fingernail clams is proportional to the increase in water temperature. All large clams were maintained in a potassium concentration of .039 mg/l during the experiments in order to maintain normal ciliary activity. Addition of 98 mg/l sodium cyanide at time A inhibited the cilia, even at a stimulatory temperature.

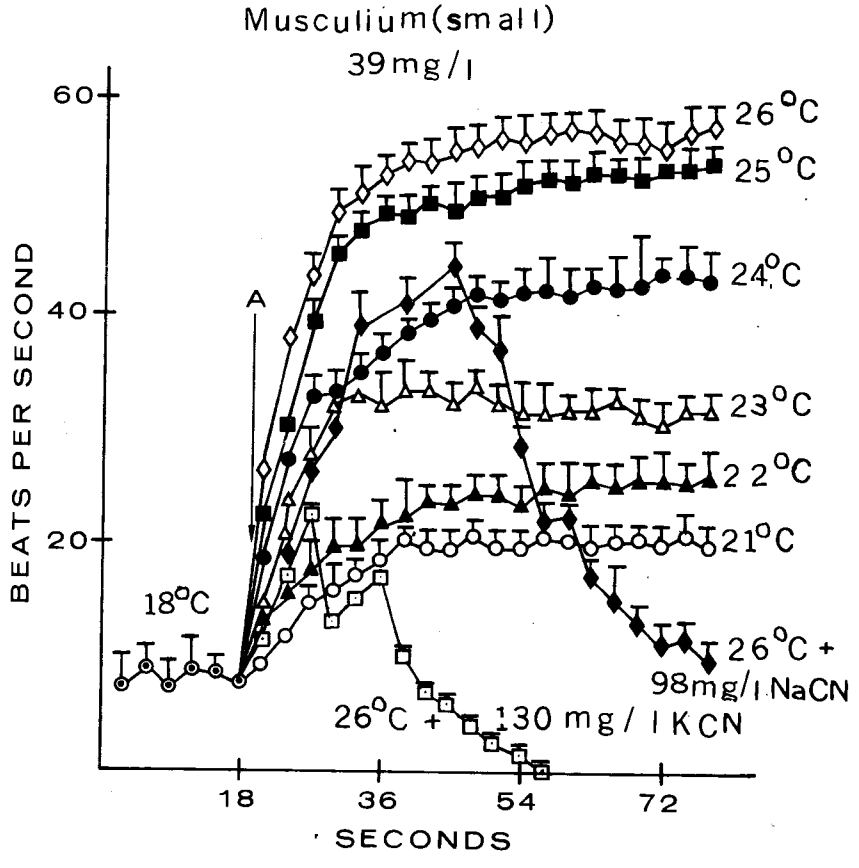



Figure 11. Beating rate of cilia on gills from small clams is proportional to the increase in water temperature. All small clams were maintained in a potassium concentration of 39 mg/l during the experiments in order to maintain normal ciliary activity. Addition of 98 mg/l sodium cyanide or 130 mg/l potassium cyanide at time A gradually inhibited the cilia, even at a stimulatory temperature.

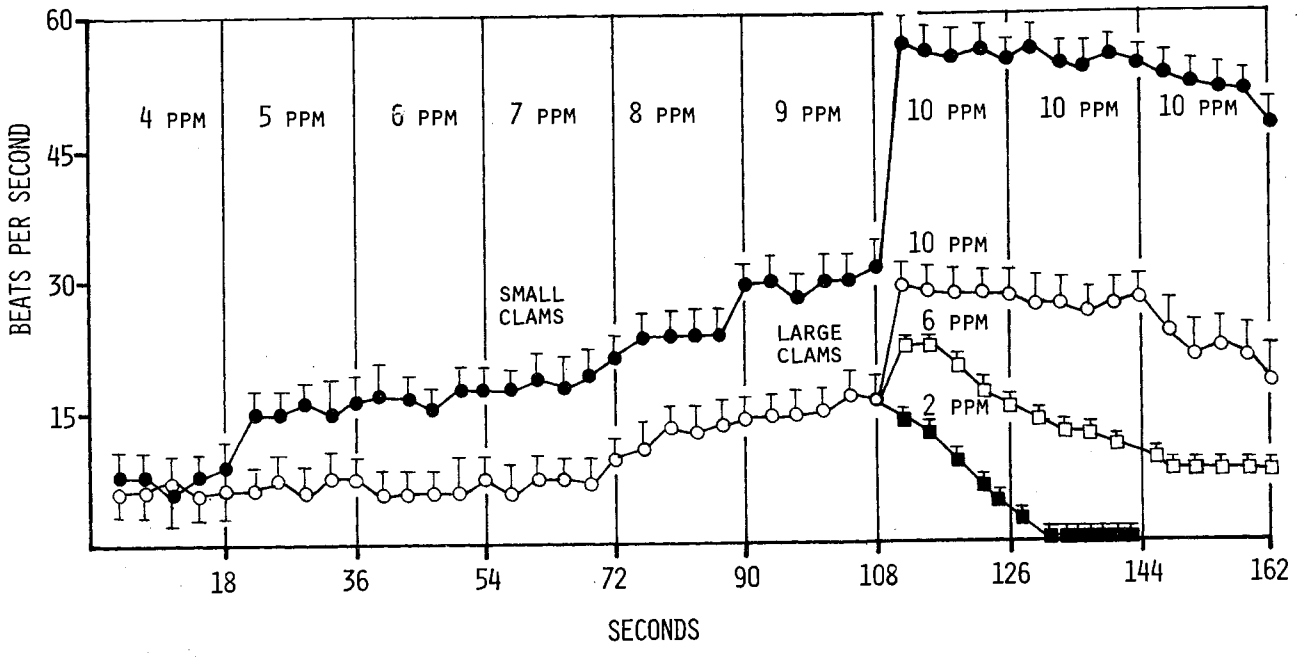



Figure 12. The ciliary beating rate of gills from large and small fingernail clams increased as the oxygen concentration of the water increased. The ciliary beating rate of both large and small clams rapidly declined to 0 in water containing 2 mg/l or less oxygen. (The response of small clams to decreased oxygen is not plotted for the sake of clarity.)

determine how long intact fingernail clams could resist dissolved oxygen concentrations of 2 parts per million or lower. Fingernail clams are found in the Des Plaines River, where the oxygen concentrations probably reach low levels due to oxygen-demanding wastes (personal communication, October 15, 1976, Mr. Thomas Butts, Chemist, Water Quality Section, Illinois State Water Survey).

A common feature of the anatomy of many species of clams is a pair of palps located near the mouth (Figure 5). The palps are oxygen-sensing organs, which apparently stimulate the lateral cilia to beat faster when the oxygen content of the water is increased. When the palps are removed, the gill

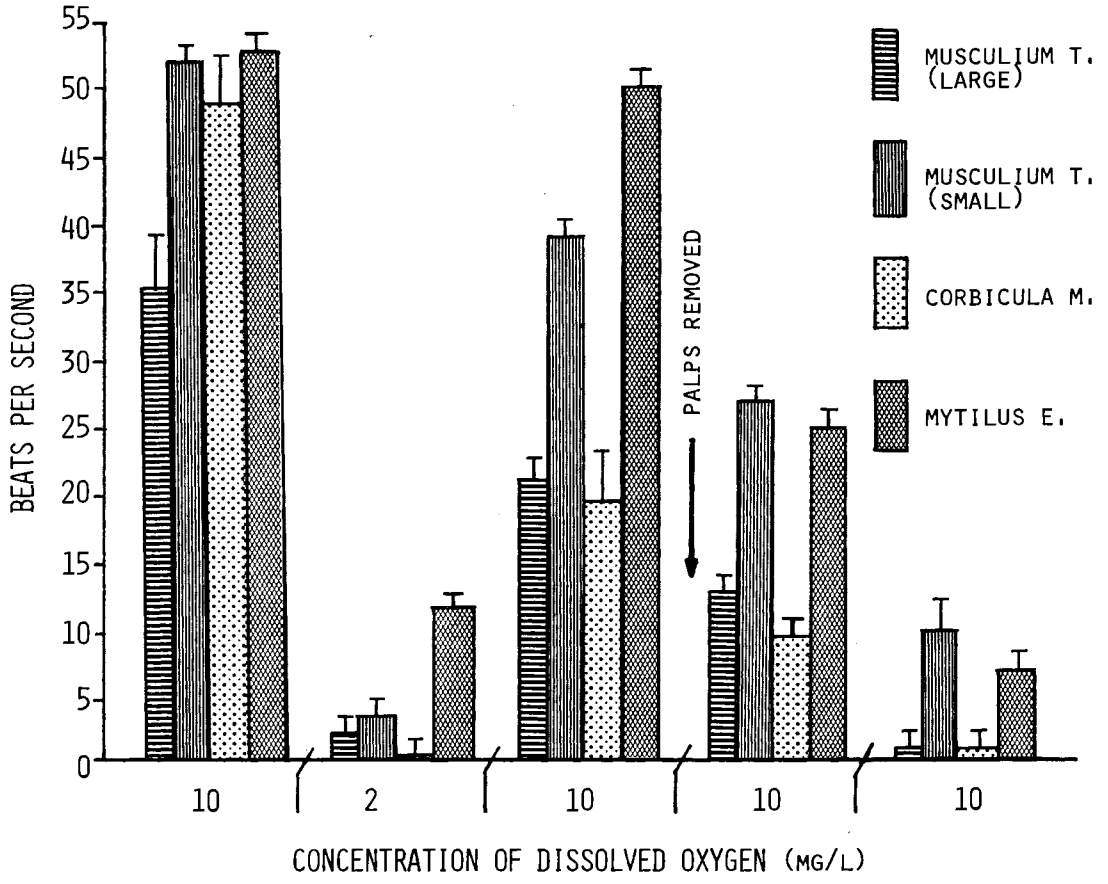



Figure 13. The ciliary beating rate of gills from large and small fingernail clams (Musculium transversum), Asiatic clams (Corbicula manilensis), and the blue mussel (Mytilus edulis) declined when the oxygen level was reduced from 10 to 2 mg/l, and recovered when the oxygen level was restored to 10 mg/l. When the oxygen-sensing palps were removed, the gills responded as though they were in oxygen-deficient water, although the ambient oxygen concentration was maintained at 10 mg/l.

preparations behave as though they were in water containing little dissolved oxygen, even though oxygen levels in the water are maintained at 10 parts per million (Figure 13).

Response of Clams to Sodium Nitrate and Sodium Sulfate

Concentrations of nitrite-nitrate, sulfate, and total dissolved solids in the Illinois River were greater than in the Mississippi River (see Tables 2-4). The purpose of exposing gill preparations to sodium nitrate and sodium sulfate

was to determine whether any of the above three factors could have affected fingernail clams. Moreover, the metals lead, copper, and zinc were added in the form of nitrates or sulfates, so it was important to determine the relative contribution, if any, of the sulfate and nitrate portion of the salts to the toxicity of the solutions.

Table 5 shows that the highest concentrations of sodium nitrate and sodium sulfate tested had no effect on the ciliary beating rates of gills from large or small clams. The concentrations of the metals are given as gram atomic weights, and are converted to mg/l as follows: sodium ($10^{-3}$ x the gram atomic weight of sodium, or 22.9 mg/l), nitrate ($10^{-3}$ x the gram ionic weight of nitrate, or 62.0 mg/l), and sulfate (.5 x $10^{-3}$ x the gram ionic weight of sulfate, or 48.0 mg/l). The Illinois Environmental Protection Agency expresses nitrate-nitrite concentrations as nitrogen, in mg/l. The maximum nitrate concentration we tested, 62.0 mg/l, is equivalent to 18 mg/l as nitrogen. The maximum nitrate-nitrite concentration (as nitrogen) shown in Table 5 for the water quality sampling stations in the Illinois River is 7.6, so nitrate concentrations in the river certainly were not high enough in 1975 to impair the ciliary activity of fingernail clams. The maximum sulfate concentrations at the sampling stations in the Illinois River ranged from 101 to 155 (Table 5), considerably above the maximum sulfate concentration we tested, so sulfate cannot be ruled out as a factor which might affect fingernail clams in the river.

The total dissolved solids (TDS) concentration in the Illinois River (Table 5) is determined by measuring the specific electrical conductivity of the water (in micromhos/cm) and multiplying by 0.6 (Illinois Environmental Protection Agency, 1976: ii). The maximum conductivity reported for the Illinois River in 1975 can be computed by dividing the maximum total dissolved

Table 5. Effects of Sodium Nitrate, Sodium Sulfate, Lead Nitrate, Copper Sulfate, and Zinc Sulfate on the Average Ciliary Beating Rate of the Gills of Musculium transversum.[a]

| Gram Atomic Weight of Metal per Liter | Average Rate of Beating (Beats/Sec.) Sodium Nitrate | Lead Nitrate | Sodium Sulfate | Copper Sulfate | Zinc Sulfate |
|---|---|---|---|---|---|
| | Small Clams (≤ 5.0 mm maximum shell length) | | | | |
| 0 (control) | 10.8±0.9 [b] | 11.5±0.6 | 12.1±0.9 | 13.1±0.5 | 12.6±0.4 |
| $10^{-12}$ | 10.7±0.8 | 11.3±0.4 | 12.3±0.6 | 10.5±0.4 | 10.9±0.3 |
| $10^{-11}$ | 10.9±0.5 | 10.5±0.3 | 12.1±0.2 | 7.8±0.8 | 11.2±0.1 |
| $10^{-10}$ | 10.5±0.7 | 9.8±0.2 | 11.8±0.6 | 7.0±0.1 | 10.6±0.1 |
| $10^{-9}$ | 10.6±0.8 | 6.8±0.2 | 11.6±0.7 | 6.1±0.5 | 7.3±0.3 |
| $10^{-8}$ | 9.9±0.9 | 6.1±0.3 | 11.3±1.2 | 4.1±0.2 | 5.5±0.1 |
| $10^{-7}$ | 10.1±0.8 | 4.2±0.2 | 11.5±0.9 | 2.1±0.1 | 5.1±0.1 |
| $10^{-6}$ | 10.3±0.4 | 3.5±0.4 | 11.3±1.1 | 1.8±0.3 | 2.0±0.3 |
| $10^{-5}$ | 10.5±0.6 | 2.7±0.6 | 11.6±0.2 | 1.2±0.6 | 1.0±0.2 |
| $10^{-4}$ | 10.9±0.5 | 1.8±0.3 | 11.5±0.3 | 1.7±0.3 | 0.8±0.1 |
| $10^{-3}$ | 10.6±0.8 | 1.1±0.2 | 11.9±0.9 | 1.3±0.2 | 0.6±0.1 |
| | Large Clams (> 5.0 mm maximum shell length) | | | | |
| 0 (control) | 9.8±0.5 | 9.6±0.8 | 10.1±0.9 | 9.6±0.3 | 8.9±1.5 |
| $10^{-12}$ | 9.7±0.8 | 9.1±0.6 | 9.8±0.2 | 8.3±0.3 | 4.3±0.5 |
| $10^{-11}$ | 9.6±0.7 | 8.1±0.4 | 9.9±0.4 | 6.1±0.2 | 3.8±0.8 |
| $10^{-10}$ | 10.1±0.3 | 5.3±0.3 | 10.3±0.2 | 2.1±0.1 | 3.2±0.7 |
| $10^{-9}$ | 10.3±0.2 | 2.1±0.1 | 10.6±0.3 | 1.2±0.1 | 2.1±0.9 |
| $10^{-8}$ | 9.6±0.3 | 1.0±0.2 | 9.8±0.7 | 0.8±0.1 | 1.2±0.8 |
| $10^{-7}$ | 9.8±0.5 | 0.8±0.1 | 9.7±0.6 | 0.3±0.1 | 1.1±0.2 |
| $10^{-6}$ | 9.6±0.7 | 0.6±0.2 | 9.7±0.8 | 0.2±0.2 | 1.0±0.3 |
| $10^{-5}$ | 9.7±0.9 | 0.7±0.3 | 9.8±0.2 | 0.3±0.3 | 0.8±0.3 |
| $10^{-4}$ | 9.8±0.3 | 0.4±0.1 | 10.3±0.3 | 0.1±0.1 | 0.2±0.2 |
| $10^{-3}$ | 10.1±0.6 | 0.3±0.1 | 10.6±0.4 | 0.2±0.1 | 0.3±0.1 |

[a] Each point is an average of 14 gills and 12 readings per gill, or a total of 168 observations.

[b] Mean ± standard deviation.

concentration of 440 mg/l by 0.6, yielding 733 micromhos/cm. We did not measure the conductivity of our test solutions. It is possible to compute the approximate conductivity of solutions using methods given in Sawyer and McCarty (1967: 184-186) and tables in the Handbook of Chemistry and Physics (1962: 2692-2696). For example, the amount of sodium sulfate added to the strongest test solution would produce an approximate conductance of 111 micromhos/$cm^2$. This does not include the contribution made to total conductance by other ions in the solution, but it indicates that the ionic strength of our test solutions was probably below the average and maximum ionic strength of the Illinois River.

Response of Clams to Sodium Cyanide and Potassium Cyanide

Figure 10 shows that the beating rate of cilia from the gills of large clams was inhibited by a cyanide concentration of 98 mg/l (as CN, not as NaCN), even at a temperature of 25 C, which would normally stimulate the cilia.

Figure 11 shows that it took longer for a cyanide concentration of 98 mg/l to inhibit the ciliary beating rate of small clams than it did for large clams. A higher concentration (130 mg/l as CN) inhibited the cilia of small clams sooner, but did not entirely prevent an initial increase in the beating rate due to the warm temperature (26 C).

Additional experiments would have to be conducted with cyanide to determine the threshold concentration for inhibition of cilia.

Response of Clams to Lead Nitrate, Copper Sulfate, and Zinc Sulfate

Since nitrate and sulfate did not have any effects on the ciliary beating rates of gill preparations, the marked effects obtained with solutions of

lead nitrate, copper sulfate, and zinc sulfate are attributable to the metals. Table 5 reports concentrations of the metals as gram atomic weights per liter (GAW/l). The concentration or concentration range in which the ciliary beating rate was reduced to 50% of the normal level is given in milligrams per liter (mg/l) or micrograms per liter (μg/l) below:

| | Large Clams | Small Clams |
|---|---|---|
| Lead | .02 μg/l | 2-20 μg/l |
| Copper | .0006 μg/l | .006-.06 μg/l |
| Zinc | .00006 μg/l | .06-.6 μg/l |

A concentration range is given in cases where the lower concentration produced less than a 50% reduction in the ciliary beating rate and the next higher concentration produced more than a 50% reduction. The concentration which would cause a 50% reduction would lie between the two concentrations actually tested.

The concentrations which caused 90% reductions in ciliary beating rates are:

| | Large Clams | Small Clams |
|---|---|---|
| Lead | 2 μg/l | 207 mg/l |
| Copper | .06-.6 μg/l | 63 mg/l |
| Zinc | .06-.65 mg/l | .65 mg/l |

The gills from large clams are much more sensitive to the metals than gills from small clams; or stated another way, the clams become more sensitive to these metals as they grow older and larger. This is the reverse of the case with fish, where the juvenile stages are usually more sensitive to toxicants than the adults.

In 1975, the average, median, and maximum concentrations of lead, copper, and zinc in the reach of the Illinois River between Creve Coeur and Havana and

in the Mississippi River at Fort Madison (Illinois Environmental Protection Agency, 1975, Volumes 2 and 4) were below the levels which would cause a 90% reduction in the ciliary beating rate of gills from small clams. Lead was below detectable limits in 3 samples taken from the Mississippi River at Fort Madison in 1975 (Illinois Environmental Protection Agency, 1975, Volume 4: 425), but averaged .01 to .02 mg/l in 3 of the 4 sampling stations on the Illinois River (Illinois Environmental Protection Agency, 1975, Volume 2: 92, 93, 103, 104). These concentrations would be sufficient to cause more than a 90% reduction in the ciliary beating rate of gills from large clams. The median concentration of copper in two of the Illinois River stations was slightly higher than in the Mississippi, and the maximum copper concentration of .02 was the same in both rivers (Illinois Environmental Protection Agency, 1975, Volume 2: 92, 93, 103, 104; Volume 4: 425). A copper concentration of .02 mg/l would cause more than a 90% reduction in the ciliary beating of gills from large clams. The maximum zinc concentration (.1 mg/l) at the Mississippi sampling station was equal to or greater than the maxima at the four Illinois River stations (Illinois Environmental Protection Agency, 1975, Volume 2: 92, 93, 103, 104; Volume 4: 425), and just within the range which caused a 90% reduction in the ciliary response of gills from large clams.

A glance at Tables 2, 3, and 4 show that several toxic metals generally occur at higher concentrations in the Illinois River than in the Mississippi. In view of the extreme sensitivity of the clam gills to copper, lead, and zinc, it appears that metals in the Illinois River could be a significant stress on adult fingernail clams. This tentative conclusion, which is based on tests with the sensitive gill preparations, should be verified with bioassays using intact fingernail clams.

Response of Clams to Potassium Chloride

Response of Gill Preparations. There was a marked difference in the response of gill preparations from small and large clams to potassium. When gills from small clams were exposed to a potassium concentration of 39 mg/1 ($10^{-3}$ M), steady ciliary beating rates were maintained for eight days (basal rate). Greater concentrations (390 to 19,500 mg/1) caused the cilia beating rate to decline (Figure 14). Concentrations ranging from 3.9 to 0.0039 mg/1

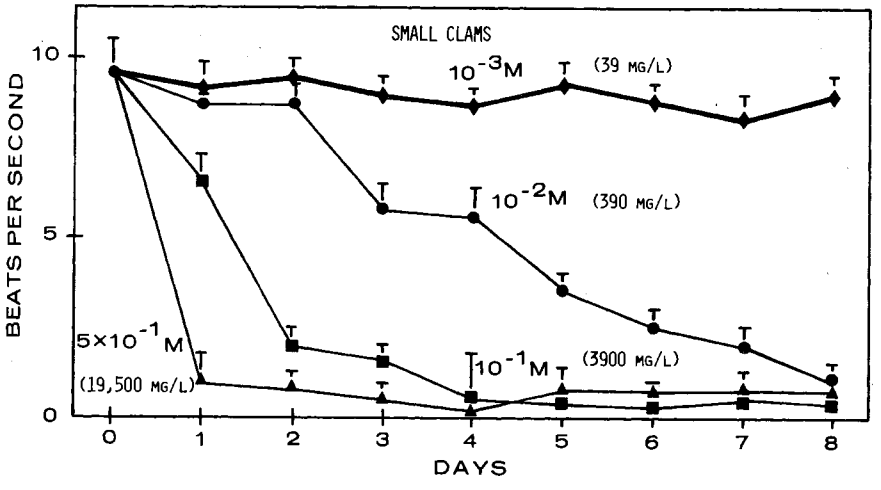



Figure 14. Ciliary beating response of gills from small clams. A potassium concentration of 39 mg/1 maintains a basal rate; higher concentrations are cilio-inhibitory.

caused a temporary increase in beating rate for three to four days, followed by a return to nearly the basal rate or slightly above (Figures 15 and 16). Lower concentrations ranging from 0.00039 to 0.000039 mg/1 caused a greater increase in beating rate for one to four days, followed by a decline below the basal rate. Finally, the lowest concentration tested, 0.0000039 mg/1, failed to excite the cilia or to maintain the basal rate (Figure 15).

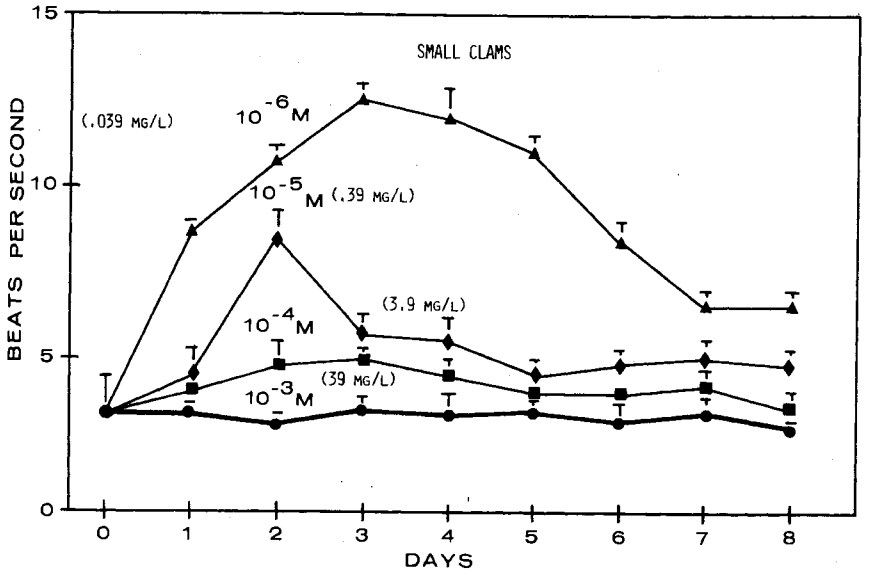



Figure 15. Ciliary beating response of gills from small clams. A potassium concentration of 39 mg/l maintains a basal rate (dashed line), while lower concentrations (3.9 and 0.039 mg/l) temporarily excite the cilia.

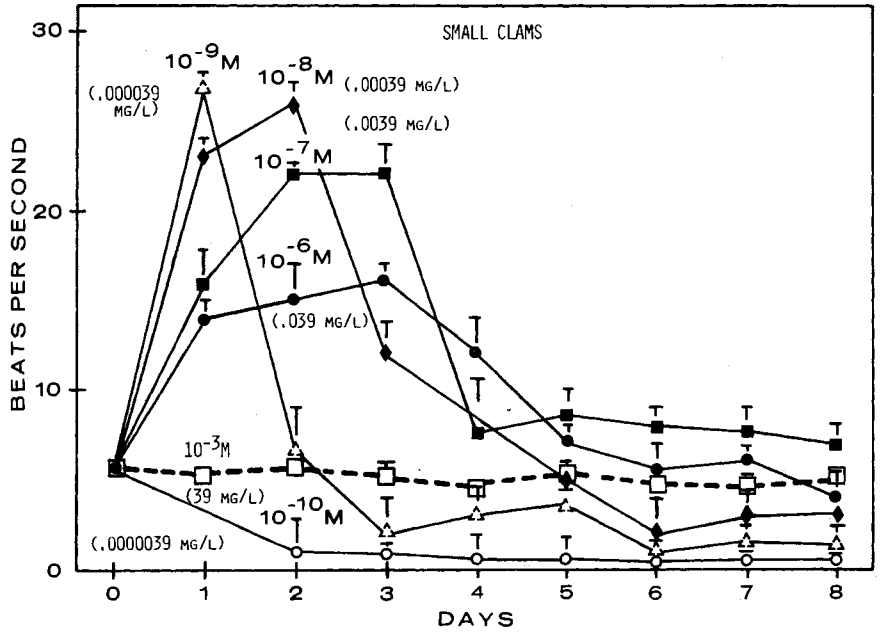



Figure 16. Ciliary beating response of gills from small clams. Continuous exposure of the gills to potassium concentrations of 0.039 and 0.0039 mg/l causes an increase in ciliary beating rate for four days, followed by a return to nearly the basal rate (dashed line) or slightly above. Potassium concentrations of 0.00039 and 0.000039 mg/l cause a greater increase in ciliary beating rate for one to three days, followed by a decline below the basal rate. The lowest concentration, 0.0000039 mg/l, fails to maintain a basal rate.

The same pattern of response to potassium occurred in large clam gills as in small clam gills, but at much lower concentrations (Figures 17 and 18).

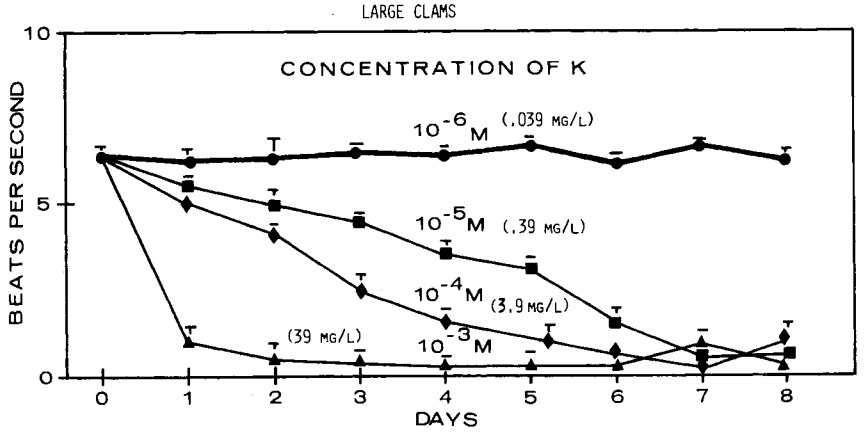



Figure 17. Ciliary beating response of gills from large clams. A potassium concentration of 0.039 mg/l maintains a basal rate; higher concentrations are cilio-inhibitory.

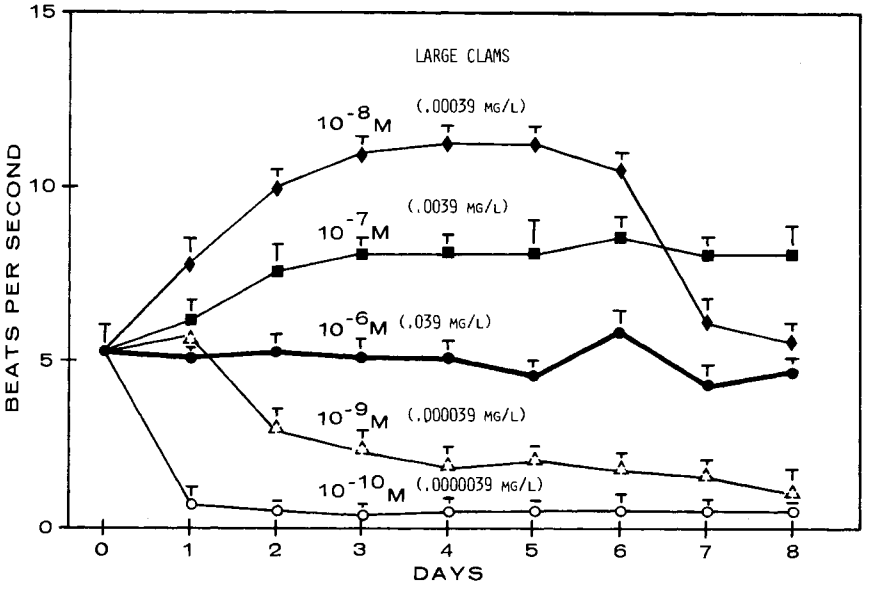



Figure 18. Ciliary beating response of gills from large clams. Potassium concentrations of 0.0039 and 0.00039 mg/l excite the cilia; while 0.000039 and 0.0000039 fail to maintain the basal rate.

For example, basal ciliary beating rates in large clam gills were maintained in 0.039 mg/l potassium ($10^{-6}$ M). Higher concentrations (0.39 to 39 mg/l) caused cilio-inhibition, lower concentrations (0.00039 to 0.0039 mg/l) caused cilio-excitation, and still lower concentrations (0.0000039 to 0.000039 mg/l) caused beating rates to decline almost to zero.

Figure 19 shows that ciliary beating rates of gills from both large and small clams declined when the gills were exposed to molluscan saline solution containing no potassium for four days. When potassium was added to the solution, the gills from small clams showed a recovery pattern, while gills from large clams did not.

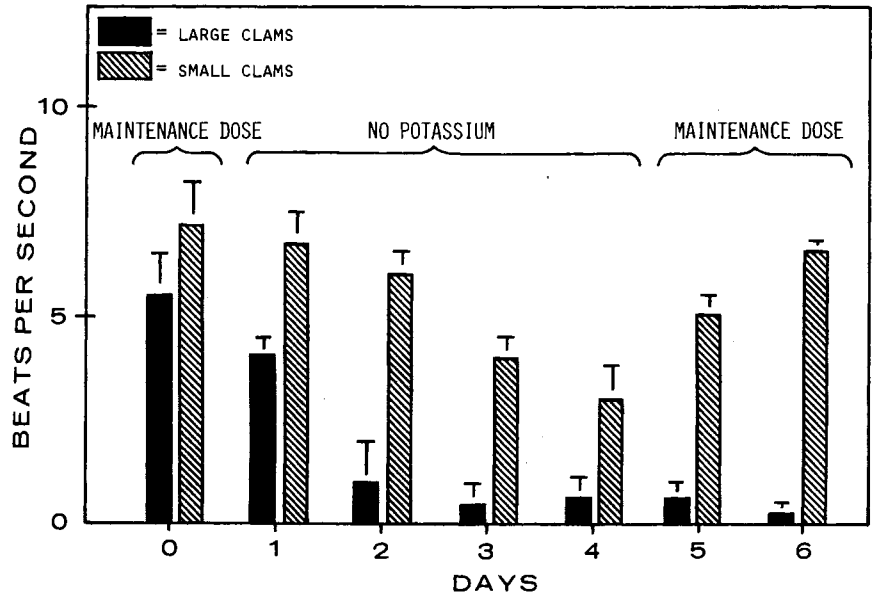



Figure 19. Ciliary beating rate of gills from large clams fails to recover from inhibiting effect of potassium withdrawal. Gills from small clams do recover.

Gills from large and small clams were kept in molluscan saline solution containing the potassium level required for maintenance of basal ciliary beating rates, then exposed to a lower potassium level (0.00039 mg/1) which was cilio-excitatory for both. Figure 20 shows that gills from large clams lagged considerably behind gills from small clams in response to the potassium reduction.

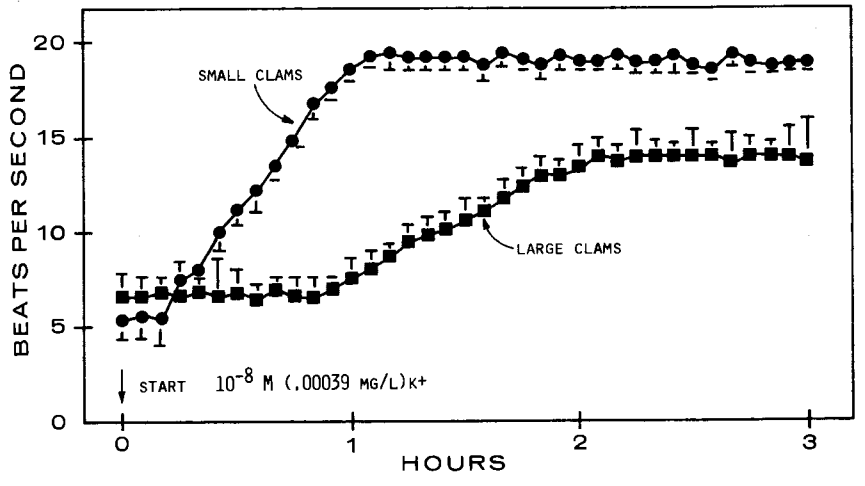



Figure 20. Gills from large clams show a greater lag than gills from small clams in response to a cilio-excitatory level of potassium.

Acute Response of Intact Clams to Potassium Chloride. Death was the response used in the acute bioassays. The acute toxicity curve indicated that potassium was a slow-acting toxicant. Lethal thresholds or probable lethal thresholds developed between 260 and 400 hours (tests J1, J2, and A2; Figures 21 and 22). In tests J3 and J4 (Figures 21 and 22), even after 600 hours of exposure, lethal thresholds did not develop. The only test in which

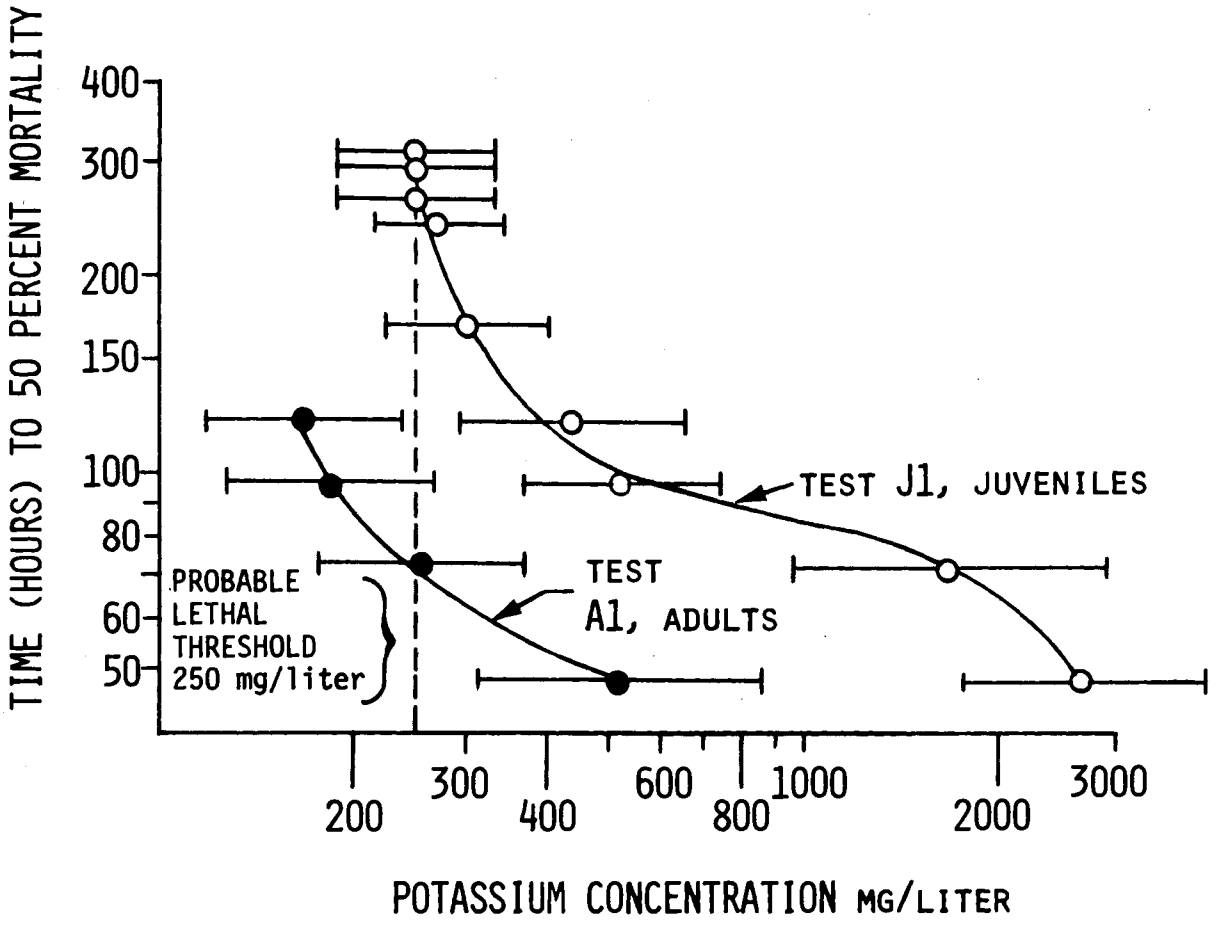



Figure 21. Comparison of acute toxicity curves, adult test A1 and juvenile test J1. The tests were conducted under the same conditions with 10 clams per concentration. The closed and open circles are concentrations which kill 50% of the clams (LC50) at different observation times during the test. The 95 percent confidence limits of each LC50 are indicated.

a valid lethal threshold developed, at a potassium concentration of 200 mg/l, was adult test A2 (Figure 22). Tests J1 (Figure 21) and J2 (Figure 22) developed probable lethal thresholds of 250 and 290 mg/l, respectively.

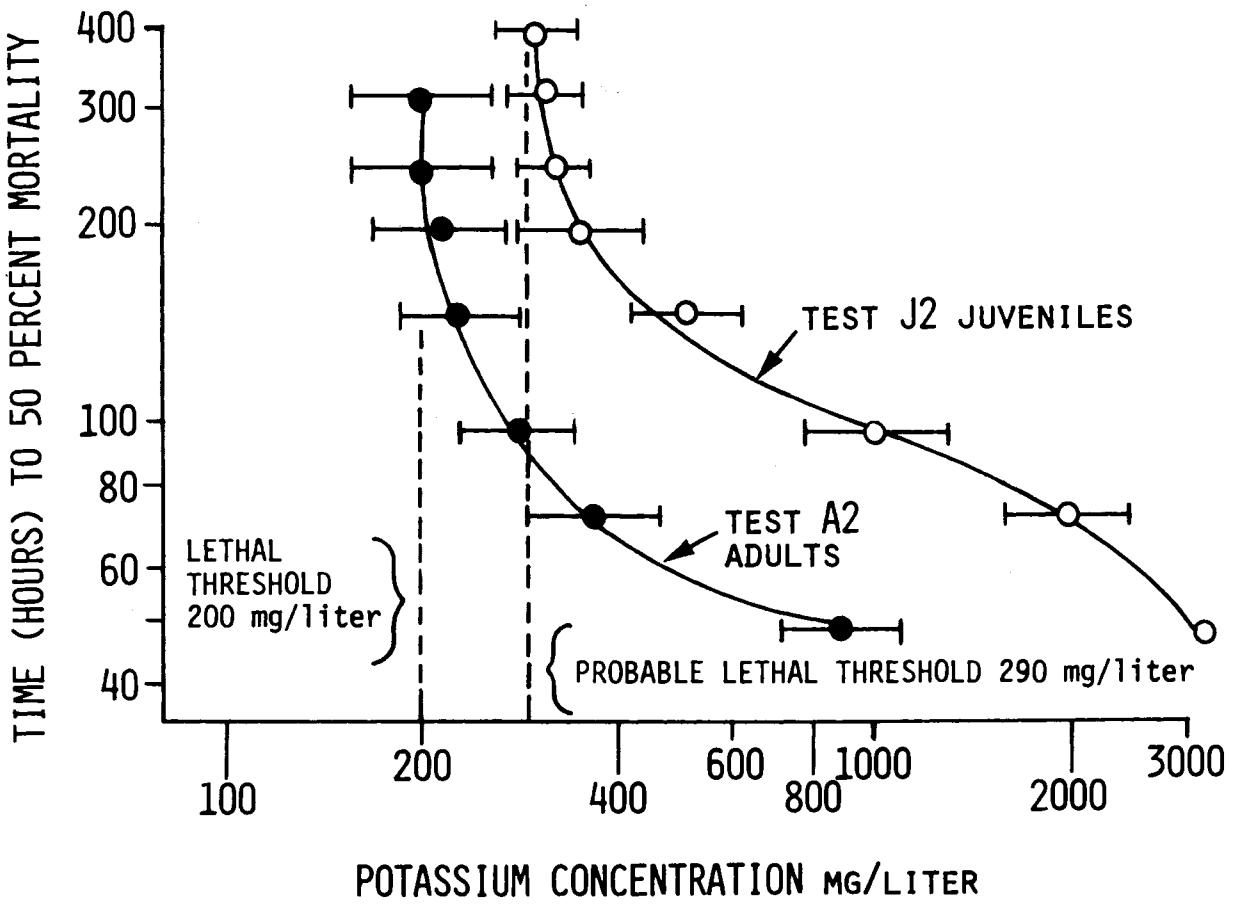



Figure 22. Comparison of acute toxicity curves of adult test A2 and juvenile test J2. The tests were conducted under the same conditions with 20 clams per concentration. The open and closed circles are potassium LC50s at different observation times during the test. The 95 percent confidence limits for each LC50 are indicated.

Toxicity curves of adult and juvenile clams under similar test conditions were compared (Figures 21 and 22) and were significantly different ($p=0.05$). Adults responded 5 to 1.6 times faster than juveniles.

Chronic Response of Intact Fingernail Clams to Potassium Chloride. Two chronic bioassays were conducted with potassium (Figures 23-26). In potassium bioassay K1, the concentrations ranged from 11.9 to 195 mg/1 potassium. There were no significant mortalities (Figure 23) or reductions in growth (Figure 24) after 42 days of continuous exposure to the highest concentration. The concentrations were increased in the second potassium bioassay K2 to produce a range from 14.3 to 275 mg/1 potassium. The only

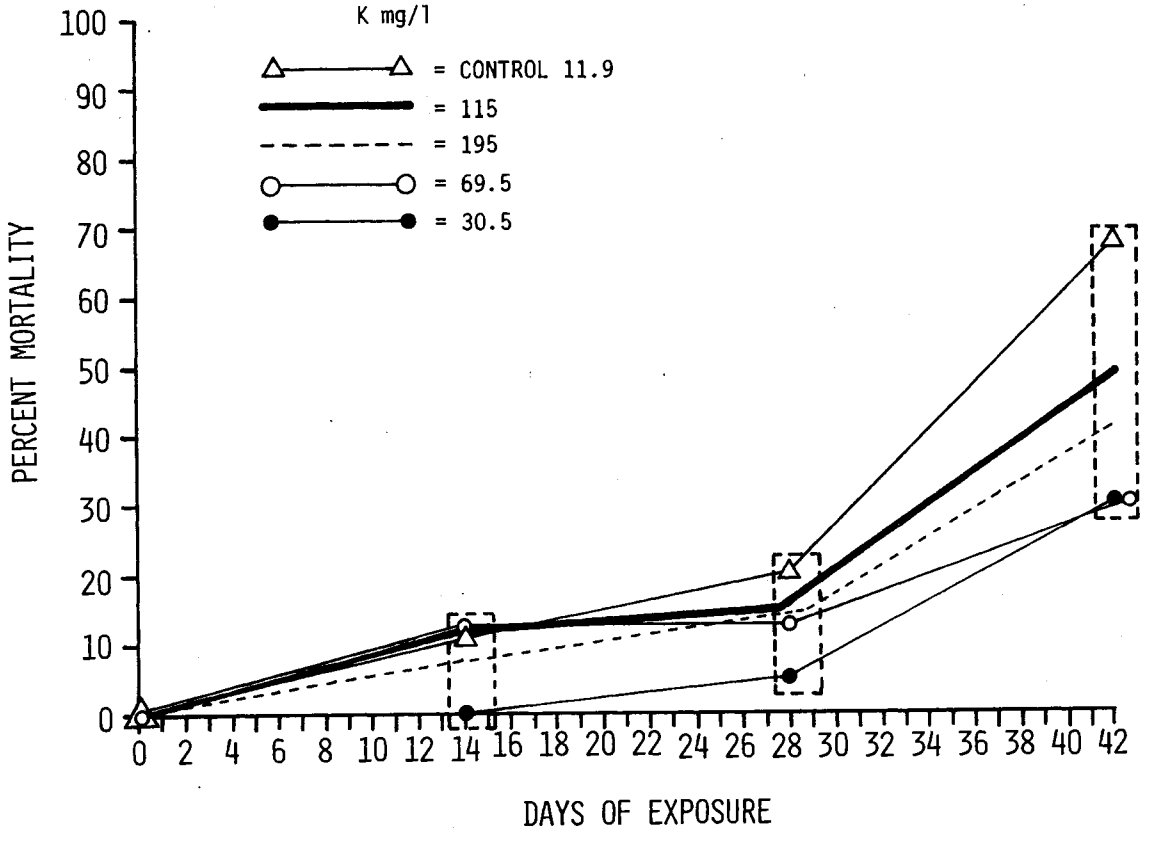



Figure 23. In the first chronic bioassay with potassium (K1), there were no significant differences in the mortality of clams exposed to the different potassium concentrations. Points which are not significantly different ($p<.05$) are included within a dashed-line box.

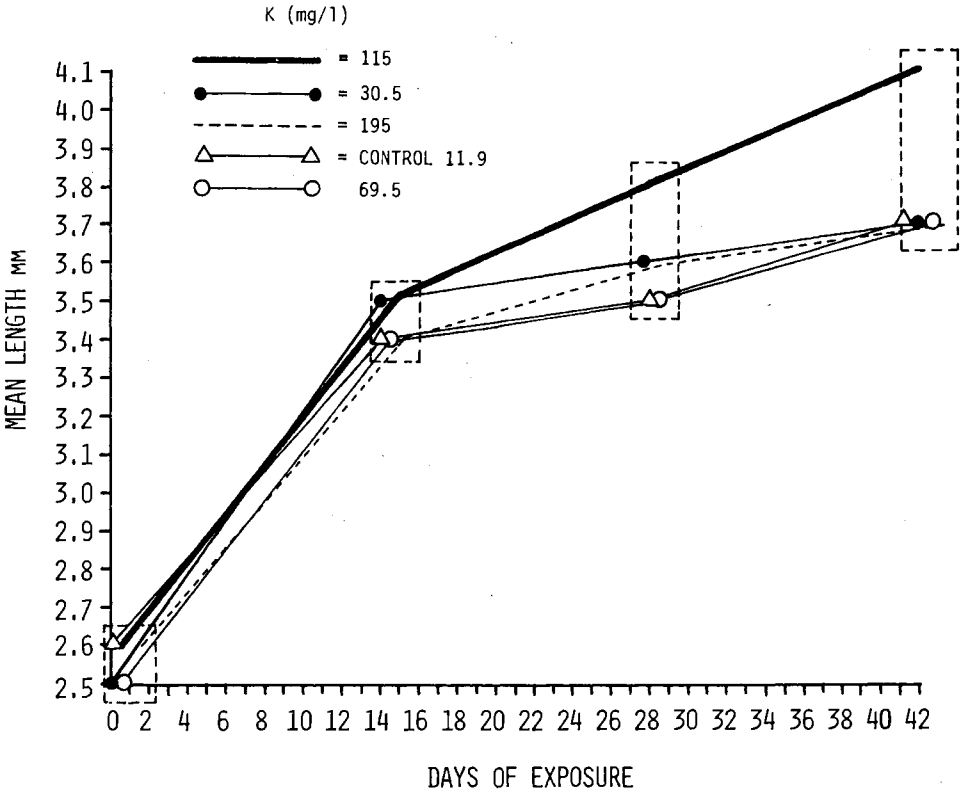



Figure 24. In the first chronic bioassay with potassium (K1), there were no significant differences in the growth (in length) of clams exposed to the different potassium concentrations. Points which are not significantly different ($p<.05$) are included within a dashed-line box.

concentration which produced significant mortalities after 14 days of exposure was the highest, 275 mg/l (Figure 25). Thus the maximum acceptable toxicant concentration (MATC) for long-term survival of fingernail clams lies between 195 and 275 mg/l potassium. Figures 24 and 26 show the effect of potassium on the growth of fingernail clams during bioassays K1 and K2, respectively. The lengths of clams surviving the potassium concentration of 275 mg/l (bioassay K2) were not used in the

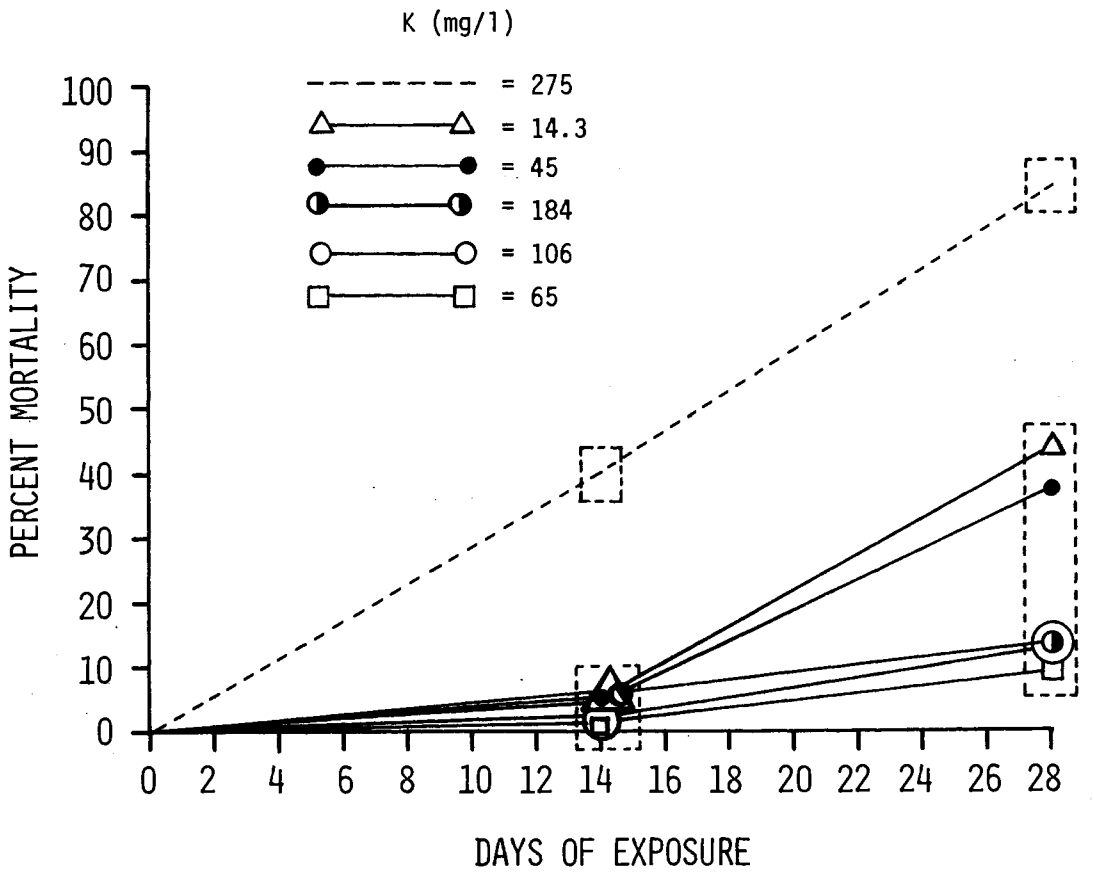



Figure 25. In the second chronic bioassay with potassium (K2) the mortality of clams exposed to 275 mg/1 was significantly greater ($p<.05$) than the mortality in the lower concentrations and in water with no added potassium.

analysis of potassium effects on growth, since the reduced number of surviving clams would cause sample bias. In comparison with the controls, none of the potassium concentrations between 45 and 184 mg/1 caused reduction in growth. On the contrary, it appears that potassium actually stimulated the growth of the clams with maximum growth occurring at 106 mg/1. Potassium apparently has no sublethal effect on the growth of fingernail clams. The effects of potassium on the reproduction of the clams was not determined, because no reproduction occurred during these bioassays, even in the controls where no potassium was added.

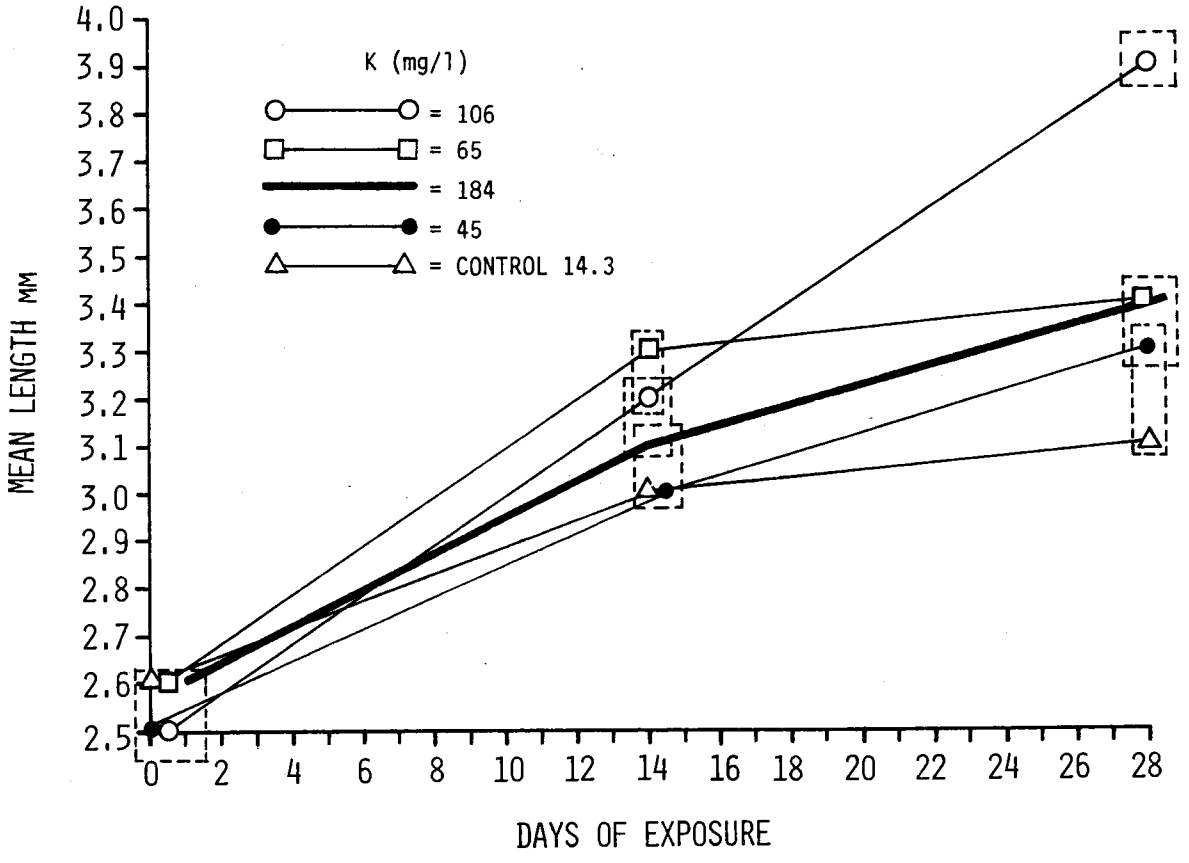



Figure 26. In the second chronic bioassay with potassium (K2), there were no significant reductions in growth of the clams exposed to the different potassium concentrations. In fact, the potassium actually stimulated growth, with maximum growth occurring at a concentration of 106 mg/1. Points which are not significantly different ($p<.05$) are enclosed within a dashed-line box.

The acute static bioassays indicate that the lethal threshold concentration (LC50) of potassium is 200 mg/1 for adult clams and probably 250-290 mg/1 for juvenile clams. The chronic bioassay data show that the maximum acceptable toxicant concentration (MATC) lies between 184 and 275 mg/1 K. In comparison to several other test organisms, M. transversum is sensitive to potassium. For example, the 96-hour LC50 for mosquite fish, Gambusia affinis, is 920 mg/1 potassium chloride (482 mg/1 potassium)(Wallen, Green, and Lasater, 1957) and for bluegill, Lepomis macrochirus, the 96-hour LC50

is 2010 mg/l potassium chloride (1054 mg/l potassium) (Anonymous, 1960). In fish toxicology, the 96-hour LC50 is comparable to a lethal threshold.

M. transversum was about as sensitive to potassium as several other invertebrates tested. The threshold concentrations for immobilization of Daphnia magna, Cyclops vernalis, and Mesocyclops leukarti are 430, 640, and 566 mg/l potassium chloride, respectively (Anderson et al., 1948). These concentrations are equivalent to 225, 336, and 297 mg/l potassium. The 96-hour LC50 for the Asiatic clam, Corbicula manilensis, is 225 mg/l potassium (Anderson et al., 1976) which indicates that the Asiatic clam may be more sensitive to potassium than M. transversum. However, a lethal threshold was not determined. Imlay (1973) found that a potassium concentration of 11 mg/l was lethal to 90 percent of 3 species of unionid clams, Actinonaias carinata, Lampsilis radiata siliquoidea, and Fusconaia flava, in 36 to 52 days of exposure; a potassium concentration of 7 mg/l was fatal to the latter 2 species in about 8 months. These species are considerably more sensitive to potassium than M. transversum.

The highest potassium concentration in 25 water samples taken near the surface of the Illinois River in 1975 was 6 mg/l potassium (Anderson, 1977), well below the lethal thresholds or probable lethal thresholds found in this study. However, before considering potassium levels in the Illinois River low enough to allow survival of the species, the microhabitat of the clam should be considered. Since the clams live in or on the sediments, they utilize water from the interface or interstitial water. Little is known concerning the chemistry of these waters in regards to equilibria with the water column and sediment. Considering that potassium concentrations as high as 250 mg/kg have been found in the sediments of the Illinois River by Mathis and Cummings (1971), it is very possible that water associated with these sediments provides

considerably higher potassium concentrations than water above the sediments. Additional research is needed in this area.

The acute static bioassay results provided information important in population dynamics. The adults respond 5 to 1.6 times faster than juveniles tested under the same conditions and showed a significantly lower lethal threshold of 200 mg/l potassium (Figure 22) when compared to the probable lethal thresholds of 250 and 290 mg/l potassium (Figures 21 and 22, respectively) for the juveniles. This is important as the reproductive portion of the population would be affected first when stressed and this could mean that death would occur before the adults could reproduce. The larger the adults grow, and the longer they live, the more young they produce. Any reduction in the longevity or growth of adults therefore would decrease production of young.

Effect of Water Hardness in Modifying Toxicity of Potassium Chloride. The effect of 2 different levels of water hardness on the response of the juvenile clams to potassium was determined (Figure 27). There was a significant difference ($p<.05$) in the response of the clams to potassium at the two levels of water hardness (Figure 27). Clams tested in the softer water, total hardness equal to 243 mg/l as $CaCO_3$ (test J2), responded faster than clams tested in the harder water, total hardness equal to 314 mg/l as $CaCO_3$ (test J3), with the toxicity curve of the soft water test approaching the vertical asymptote at 400 hours (Figure 27). In contrast, the toxicity curve of the clams tested in the harder water does not show a lethal threshold even at 696 hours.

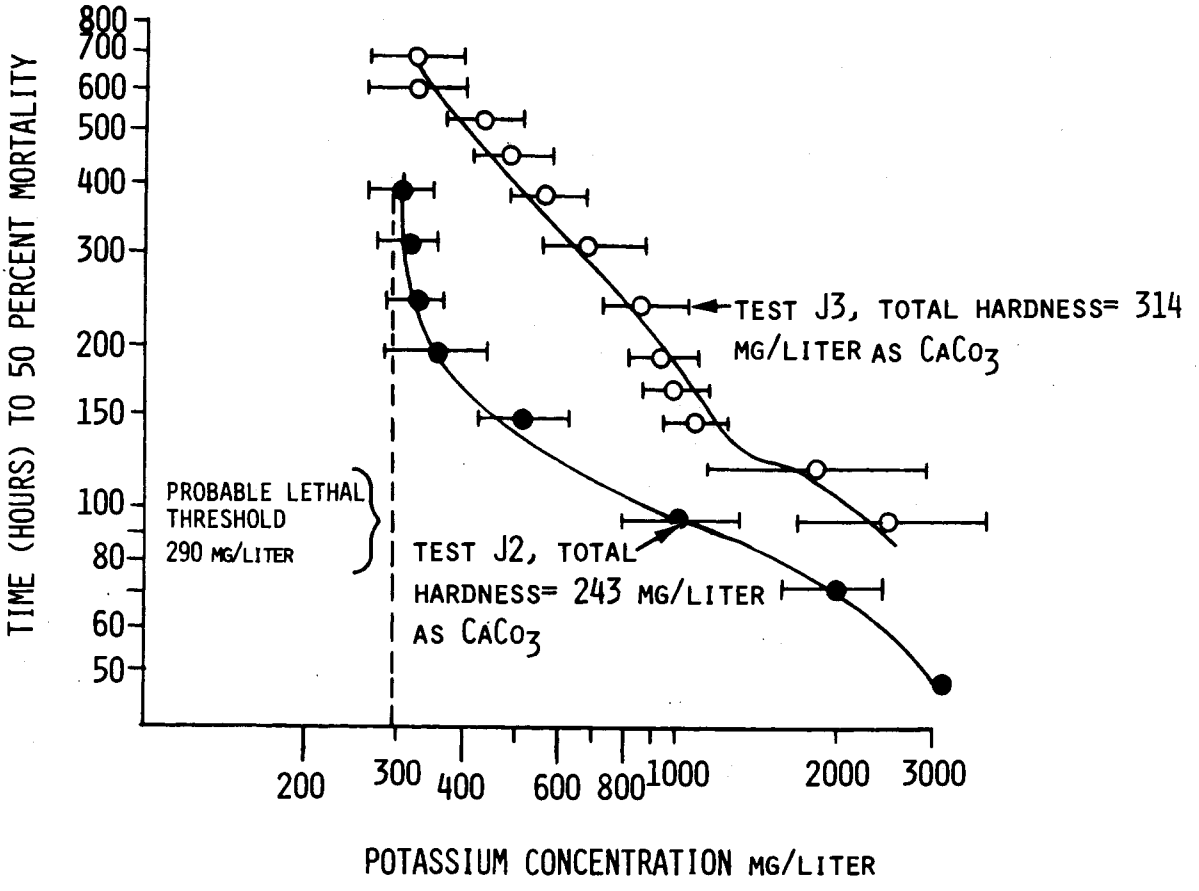



Figure 27. Clams exposed to potassium in hard water (314 mg/l as $CaCO_3$) did not die as rapidly as clams exposed to potassium in softer water (243 mg/l as $CaCO_3$).

Effect of Temperature in Modifying Toxicity of Potassium Chloride. The LC50s in the test where the water temperature was 6.5 C were significantly greater ($p<.05$) than in the test at 16.7 C, with the exception of the 96-hour LC50 (Figure 28). The toxicity curve of 16.7 C test approached the vertical asymptote in 400 hours whereas in the 6.5 C test a lethal threshold had not developed even after 648 hours (Figure 28). It did appear that a vertical asymptote was being approached after 648 hours in the test at 6.5 C, and

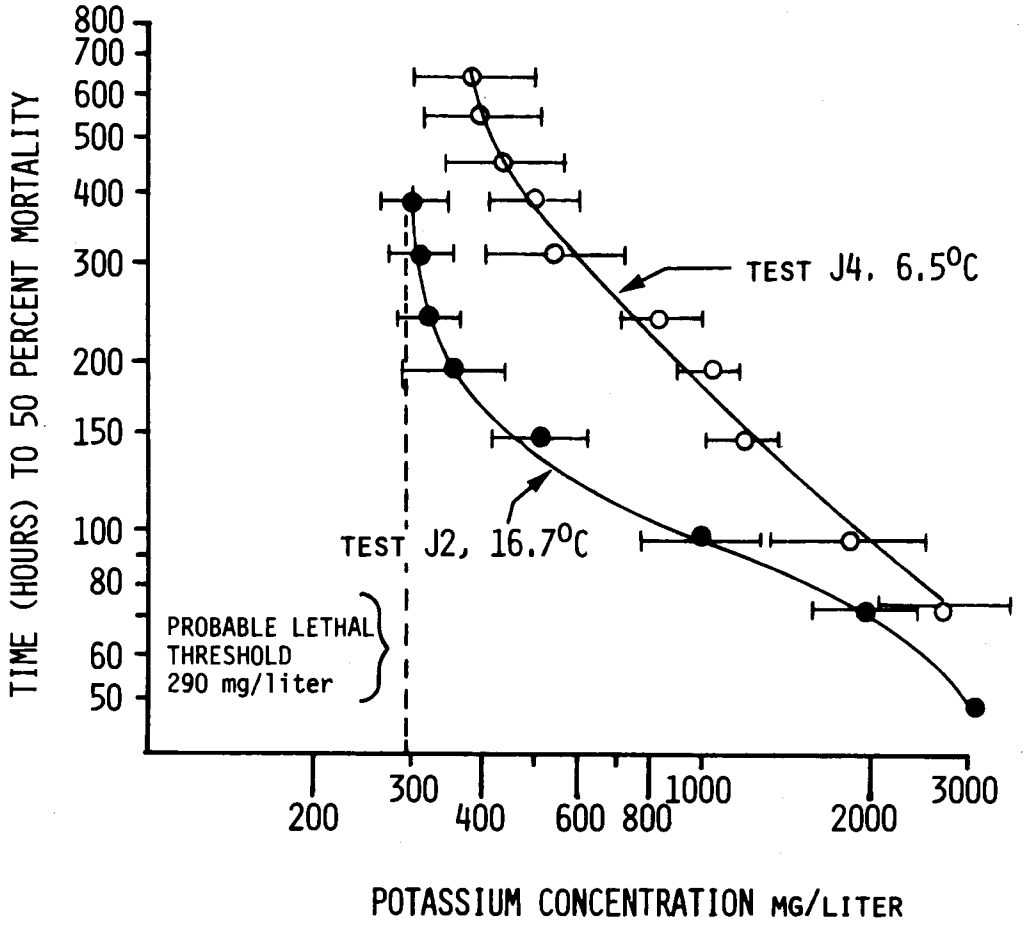



Figure 28. Juvenile fingernail clams died more rapidly in warm water than in cool water when exposed to potassium chloride, but the lethal thresholds at the two temperatures were probably almost the same.

that the asymptotic value would have been close to that in the test at 16.7 C. The cold temperature apparently slowed the rate of uptake or the rate of toxic action of potassium, but did not change the lethal threshold. This means that an agent whose toxic action was similar to that of potassium would kill clams more slowly at cold water temperatures than at warm temperatures, but that if the exposure were continued long enough, the same percentage of the population would be killed in both cases.

Response of Clams to Ammonium Chloride

Response of Gill Preparations. Figure 29 shows that gills from large fingernail clams were more sensitive to un-ionized ammonia than gills from small clams.

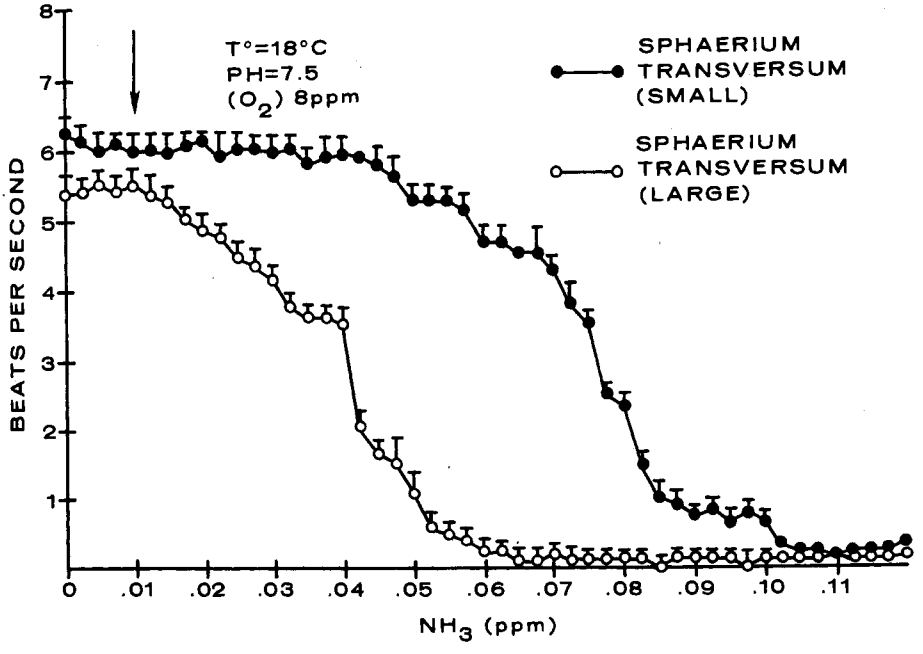



Figure 29. Ciliary beating response of gills from large and small fingernail clams to un-ionized ammonia (expressed as $NH_3$, ppm, or mg/l).

The un-ionized ammonia concentrations which induced various degrees of inhibition of the ciliary activity of gills from large and small clams are given below. The concentrations are expressed as un-ionized ammonia nitrogen, $NH_3$-N mg/l, which is the way ammonia concentrations are reported by the Illinois Environmental Protection Agency. The un-ionized ammonia values in Figure 29 were converted to $NH_3$-N by multiplying by .824.

| | 50% reduction in ciliary beating rate | 90% reduction in ciliary beating rate | complete inhibition of cilia |
|---|---|---|---|
| Gills from large clams | .03 mg/1 | .04 mg/1 | .05-.06 mg/1 |
| Gills from small clams | .06-.07 mg/1 | .08 mg/1 | .08-.09 mg/1 |

Adult fingernail clams are slightly more sensitive to un-ionized ammonia than the Asiatic clam (Corbicula manilensis), a freshwater unionid mussel (Elliptio complanata), and a marine mussel (Mytilus edulis), as can be seen by comparing Figures 29 and 30.

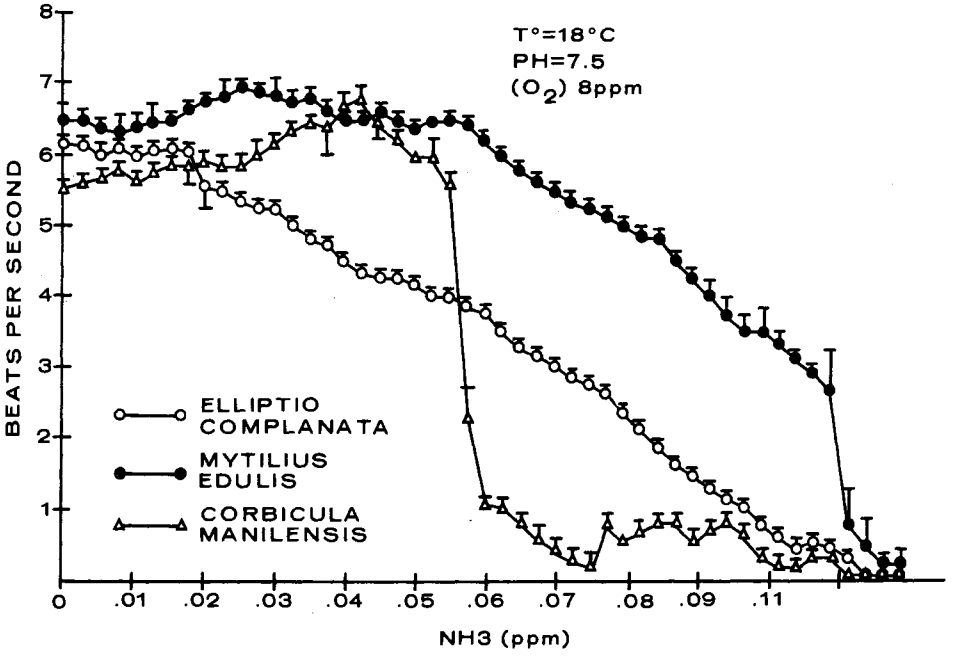



Figure 30. Ciliary beating response of gills from the Asiatic clam (Corbicula manilensis), a freshwater mussel (Elliptio complanata), and a marine mussel (Mytilus edulis), to un-ionized ammonia (expressed as $NH_3$, ppm, or mg/1). Note .11 on horizontal scale should be .10.

The dose-response curves for large and small fingernail clams display a classic sigmoid pattern (Figure 29), while the decrease in the ciliary response of the Asiatic clam is so abrupt between .05 and .06 mg/1 $NH_3$ that the curve is almost rectilinear (Figure 30). The ciliary response of Elliptio complanata decreases linearly with increasing concentration of un-ionized ammonia. The un-ionized ammonia concentrations which produced various levels of ciliary inhibition in the three species, other than fingernail clams, are given below (concentrations are expressed as un-ionized ammonia nitrogen, $NH_3$-N, mg/1):

| | 50% reduction in ciliary beating rate | 90% reduction in ciliary beating rate | complete inhibition of cilia |
|---|---|---|---|
| Elliptio complanata | .06 mg/1 | .09 mg/1 | .09-.10 mg/1 |
| Mytilus edulis | .08 mg/1 | .09 mg/1 | .09-.10 mg/1 |
| Corbicula manilensis | .05 mg/1 | .06 mg/1 | .09-.10 mg/1 |

Effect of Oxygen in Modifying Toxicity of Ammonium Chloride. Figure 31 shows that increasing the dissolved oxygen concentration in the water above the saturation concentration of 9.18 mg/1, reduces the inhibitory action of un-ionized ammonia on the cilia of the gills.

At the same $NH_3$-N concentration of .07 mg/1, the ciliary beating rate of gills from large clams was barely maintained at the highest oxygen concentration tested, 16 mg/1 (170% of saturation), Figure 32.

These results indicate that the sensitivity of the gill preparations to un-ionized ammonia increases as the oxygen content of the water decreases.

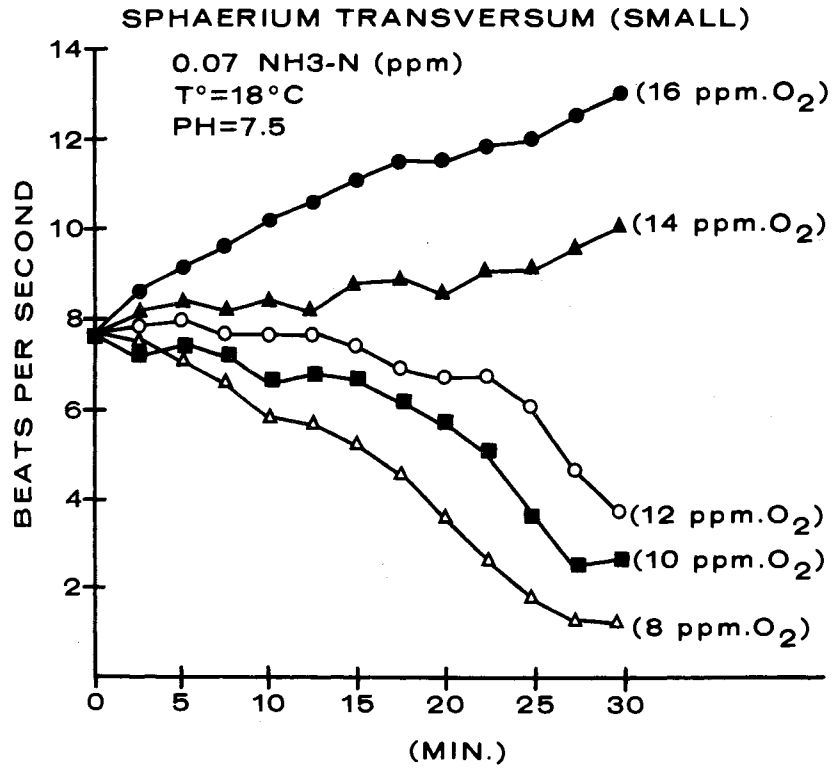



Figure 31. Ciliary beating rates of gills from small clams did not decrease when the gills were exposed to .07 mg/1 $NH_3$-N and oxygen concentrations of 14 mg/1 or more (150% or more of the oxygen saturation concentration of 9.18 mg/1).

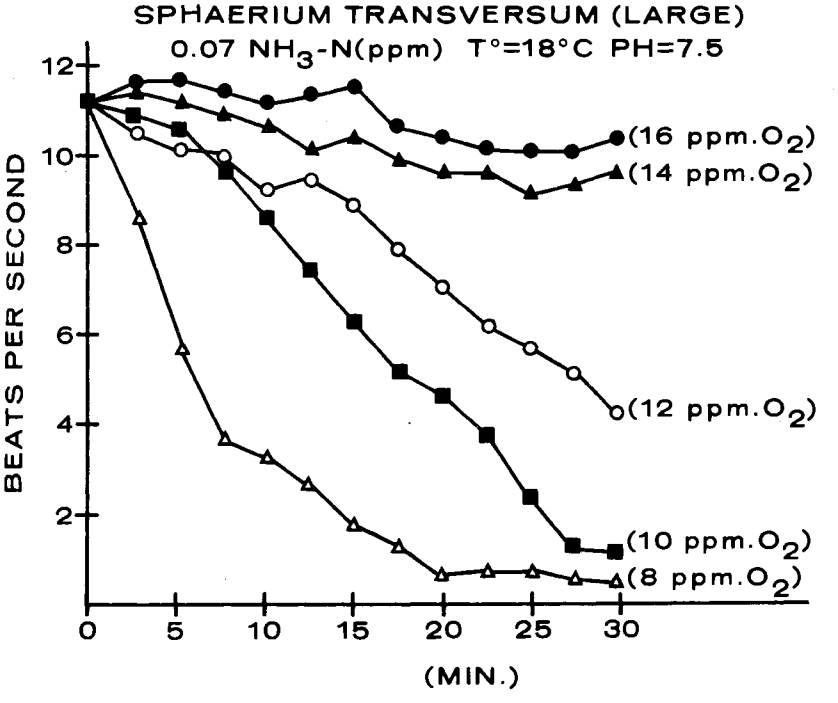



Figure 32. The ciliary beating rates of gills from large fingernail clams decreased markedly when the gills were exposed to .07 mg/1 $NH_3$-N, and oxygen concentrations below 12 mg/1 (130% of saturation). Even at the highest oxygen concentrations of 14 and 16 mg/1 (150% and 170% of saturation) the ciliary beating rates declined slightly.

Chronic Response of Intact Clams to Ammonium Chloride. Two chronic bioassays were conducted with ammonia (Figures 33-36). In chronic bioassay $NH_3$2 significant mortalities occurred in the upper 2 ammonia concentrations, 0.59 and 0.93 mg/l undissociated $NH_3$-N, after 42 days of continuous exposure to ammonia (Figure 33). This results in a maximum acceptable toxicant concentration (MATC), based on mortality, between 0.35 and 0.59 mg/l un-ionized $NH_3$-N. Growth data from the test concentrations where there was not a significant difference in mortality indicate that there was no effect on the

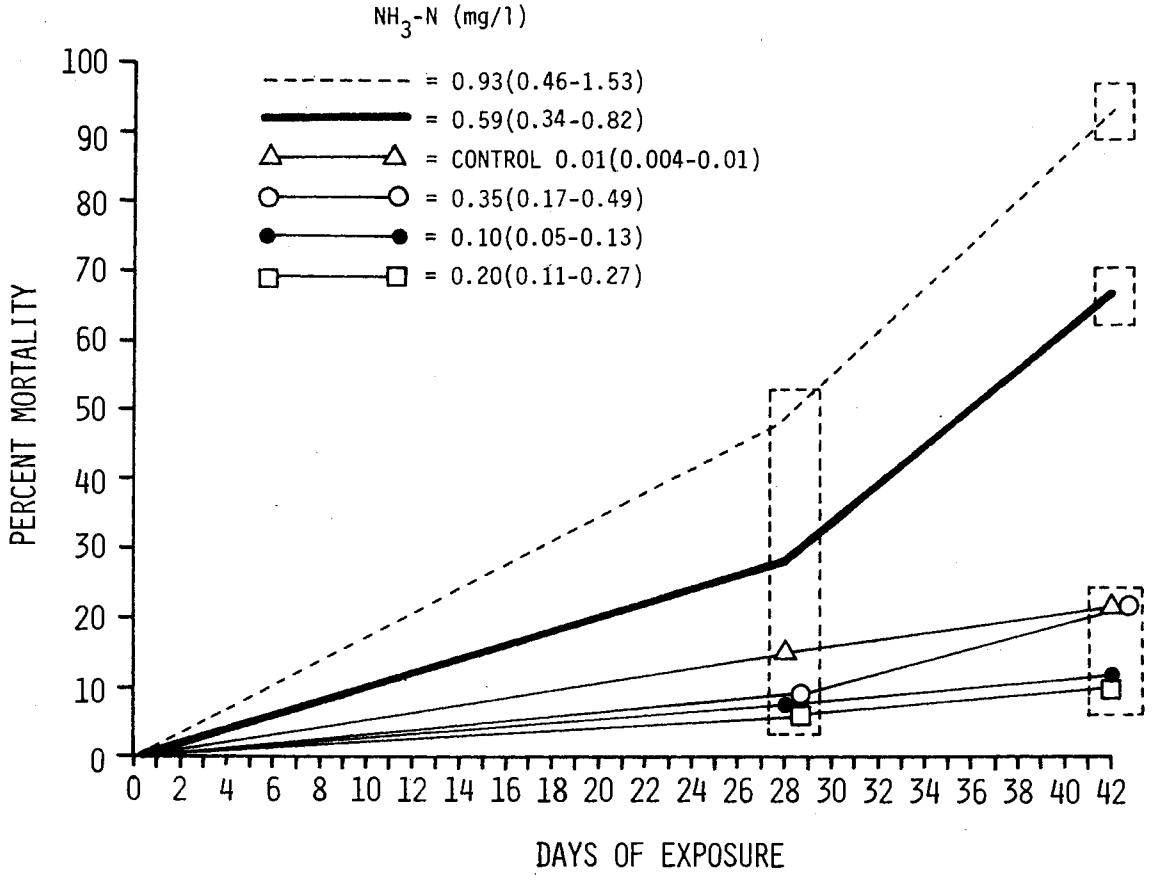



Figure 33. Un-ionized ammonia concentrations of .93 and .59 mg/l (as $NH_3$-N) caused significant ($p<.05$) mortality among fingernail clams after 42 days of exposure. All points within the same box are not significantly different ($p<.05$).

growth, e.g. growth was not reduced below that of the control (Figure 34). However, growth was not good during the experiments with a maximum growth of only 0.5 mm in 42 days. It was suspected that the slow growth was due to the small size of clams that were used at the initiation of the experiment (2.1-2.3 mm) and the possibility that the clams were born in the laboratory under stress conditions. Subsequent work showed that using clams that averaged 2.5 mm or greater in length resulted in better growth.

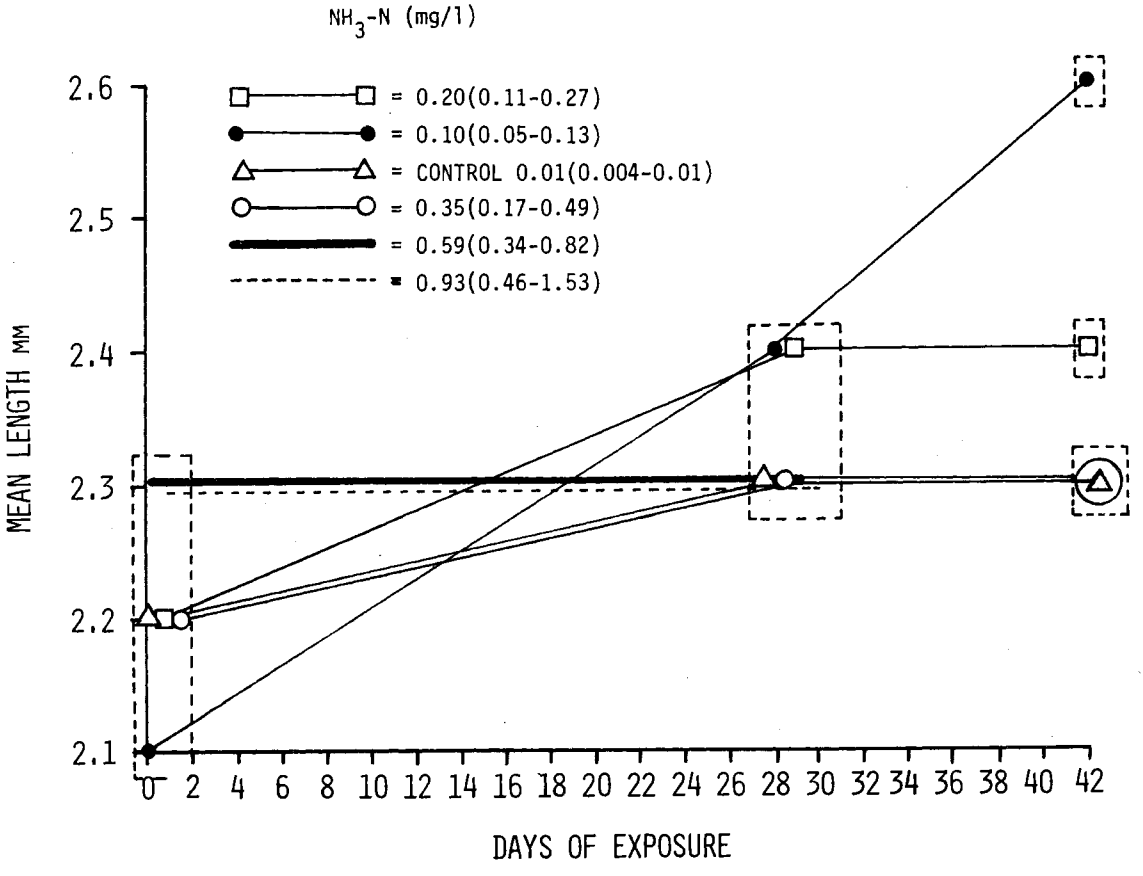



Figure 34. Results of chronic bioassay $NH_3$2. Un-ionized ammonia concentrations of 0.10 and 0.20 mg/l actually stimulated the growth of fingernail clams, presumably by stimulating the growth of bacteria upon which the clams feed. Growth at all other concentrations was not significantly different ($p<.05$) from that in the control.

A second ammonia chronic bioassay, $NH_3$3, was conducted in an attempt to obtain adequate growth data to estimate the MATC for growth. The upper concentration of un-ionized ammonia (1.20 mg/l $NH_3$-N) caused significant mortality after 14 days of exposure, and the next highest concentration (0.60 mg/l $NH_3$-N) caused significant mortality after 42 days (Figure 35). Thus the MATC, based on mortality, lies between 0.34 and 0.60 mg/l un-ionized ammonia ($NH_3$-N). These results confirmed the results obtained in test $NH_3$2.

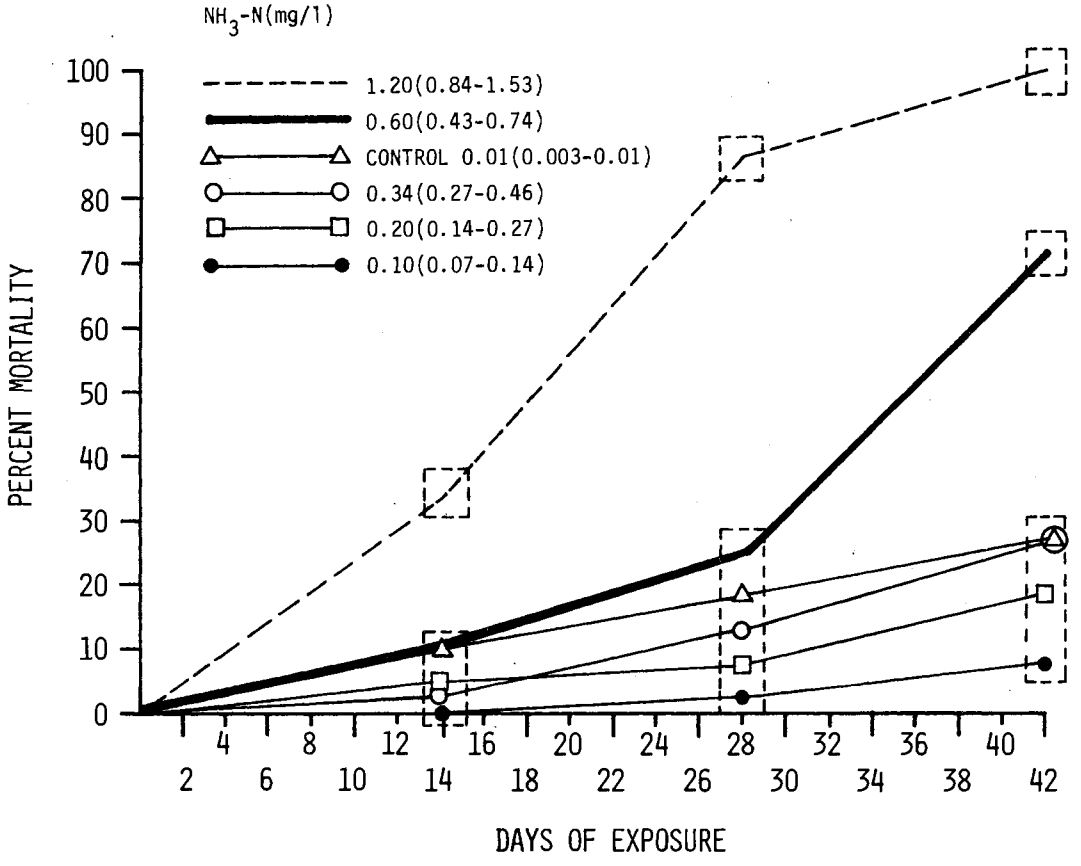



Figure 35. An un-ionized ammonia concentration of 1.20 mg/l caused significant ($p<.05$) mortality among fingernail clams after 14 days of exposure, and a concentration of 0.60 mg/l caused significant mortality after 42 days. Mortalities at the other concentrations were not significantly different from controls.

Clams in un-ionized ammonia concentrations where significant mortality occurred were not used to determine effects of ammonia on growth, because the sample might become biased if the different size classes of clams differed in their susceptibility to ammonia. For example, the experiments with the gill preparations demonstrated that gills from large clams were more sensitive to ammonia than gills from small clams. If large clams are more readily killed by ammonia than small clams, then the average size of clams in lethal concentrations of ammonia would diminish during the course of an

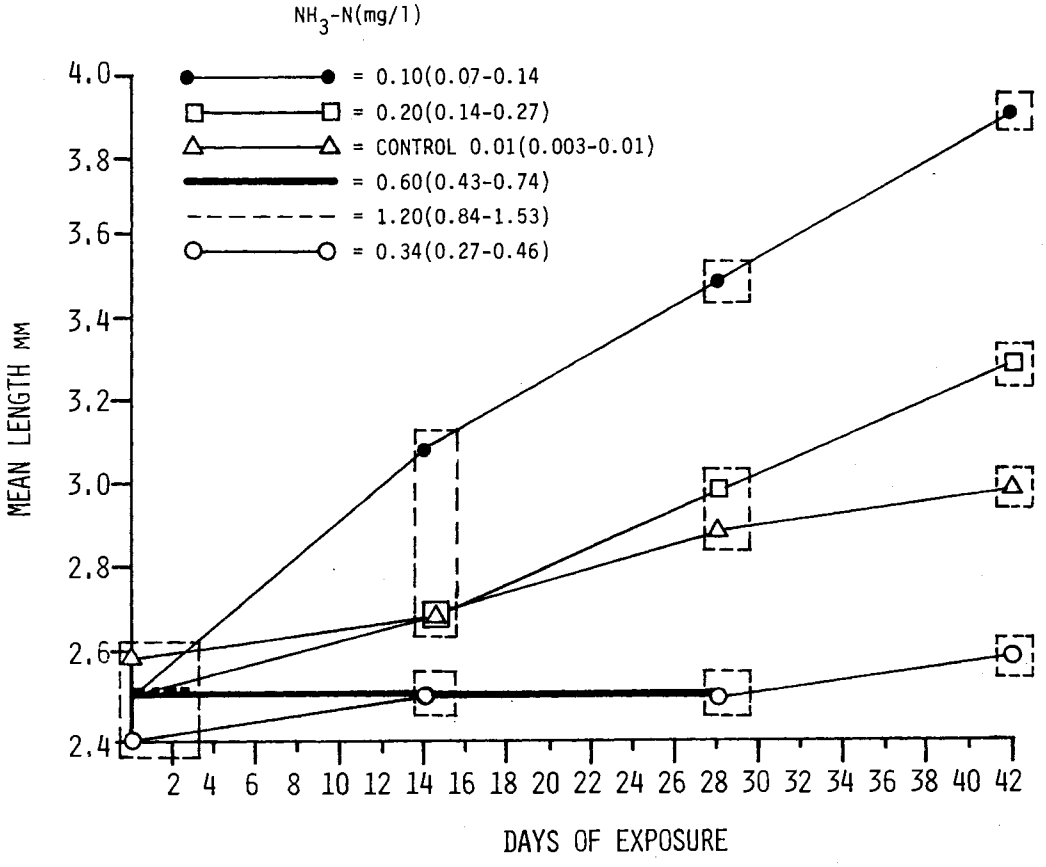



Figure 36. Un-ionized ammonia concentrations of 0.34 and 0.60 mg/l ($NH_3$-N) significantly ($p<.05$) reduced the growth of fingernail clams after 14 days of exposure. Growth of clams was significantly enhanced at lower concentrations probably due to increased growth of bacteria upon which the clams feed. All data points within the same box are not significantly different ($p<.05$).

experiment, in comparison to clams in sublethal concentrations, but the effect would be attributable to size-selective mortality, rather than to a direct effect of ammonia on growth.

Figure 36 shows that un-ionized ammonia concentrations of 0.34 and 0.60 mg/l ($NH_3$-N) significantly ($p<.05$) reduced the growth of fingernail clams after 14 days of exposure. The MATC, based on growth, lies between 0.20 and 0.34 mg/l un-ionized ammonia ($NH_3$-N).

The results of the chronic bioassays with ammonia are summarized below:

| Test No. | MATC based on mortality | MATC based on growth |
|---|---|---|
| $NH_3$2 | >0.35<0.59 mg/l $NH_3$-N | - |
| $NH_3$3 | >0.34<0.60 mg/l $NH_3$-N | >0.20<0.34 mg/l $NH_3$-N |

Effect of Sublethal Exposure to Ammonium Chloride on Subsequent Response to Stress. Fingernail clams which had been exposed for 44 days to well water containing no added ammonia or to sublethal concentrations of 0.10 and 0.20 mg/l un-ionized ammonia in test $NH_3$3 were delivered to Southern Illinois University for testing in the cilia monitoring apparatus. They were held for one week in a culture tank containing no added ammonia before their gills were removed and tested.

Figure 37 shows that chronic exposure of clams to sublethal concentrations of ammonia alters the response of their gills to potassium. The ciliary beating rate of gills from clams previously exposed to 0.1 mg/l $NH_3$-N was subsequently stimulated at potassium concentrations of $10^{-4}$ to $10^{-5}$ M (3.9-.39 mg/l), whereas gills from clams not exposed to ammonia showed maximum stimulation at potassium concentrations ranging from $10^{-5}$ to $10^{-10}$ M (.39-.0000039 mg/l). The maximum ciliary beating rate of the gills exposed to 0.1 mg/l $NH_3$-N was only 15, whereas the maximum was 28 for the unexposed group. Previous exposure

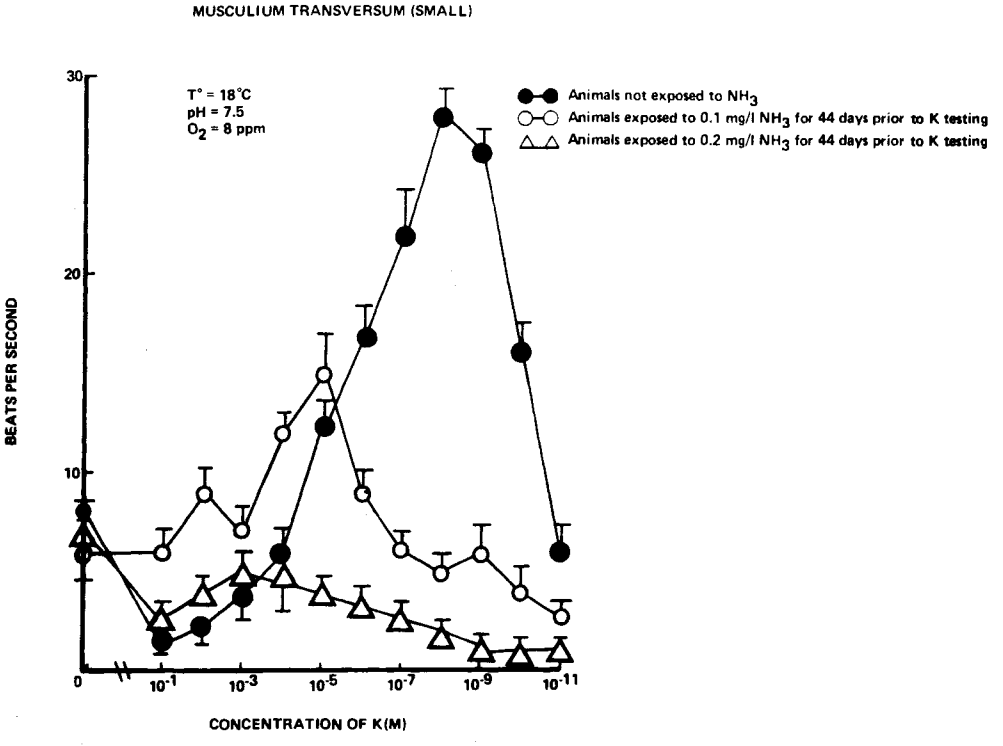



Figure 37. The ciliary beating response to potassium of gills from clams previously exposed for 44 days to sublethal $NH_3$-N concentrations of 0.1 and 0.2 mg/l was markedly altered in comparison to the response of gills from clams not previously exposed to ammonia.

to 0.2 mg/l $NH_3$-N completely blocked the stimulatory response to potassium addition. In fact, the ciliary beating rate was slightly to markedly inhibited at all potassium concentrations.

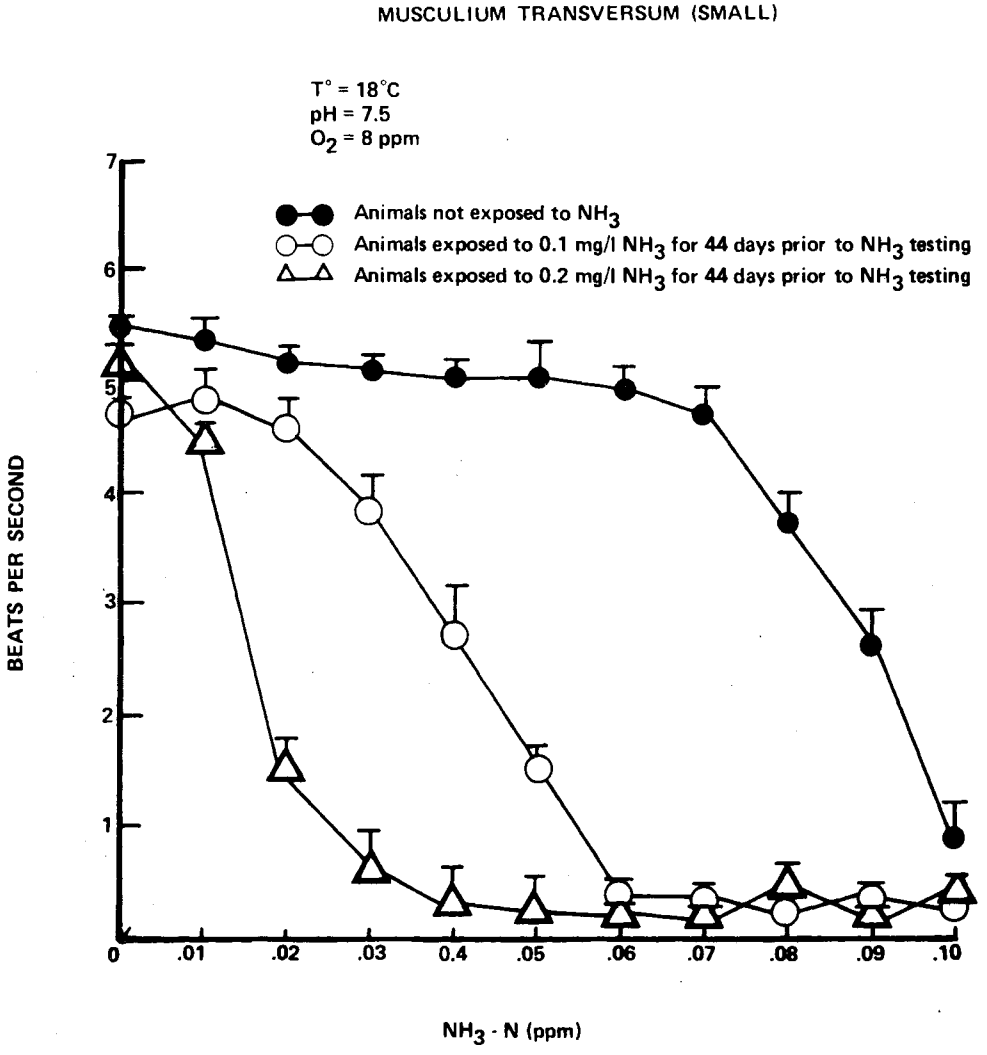



Figure 38. When clams are exposed to sublethal concentrations of un-ionized ammonia (0.1 and 0.2 mg/l $NH_3$-N) for 44 days, their gills are sensitized to subsequent additions of ammonia.

Figure 38 shows that the gills of the clams are sensitized to ammonia by previous exposure to sublethal concentrations of ammonia. A 50% reduction in the ciliary beating rate occurs at an un-ionized ammonia concentration of .09 mg/l ($NH_3$-N) in control gills not previously exposed to ammonia, at .04 mg/l in gills previously exposed to 0.1 mg/l $NH_3$-N, and at .01 to .02 mg/l in gills previously exposed to 0.2 mg/l $NH_3$-N.

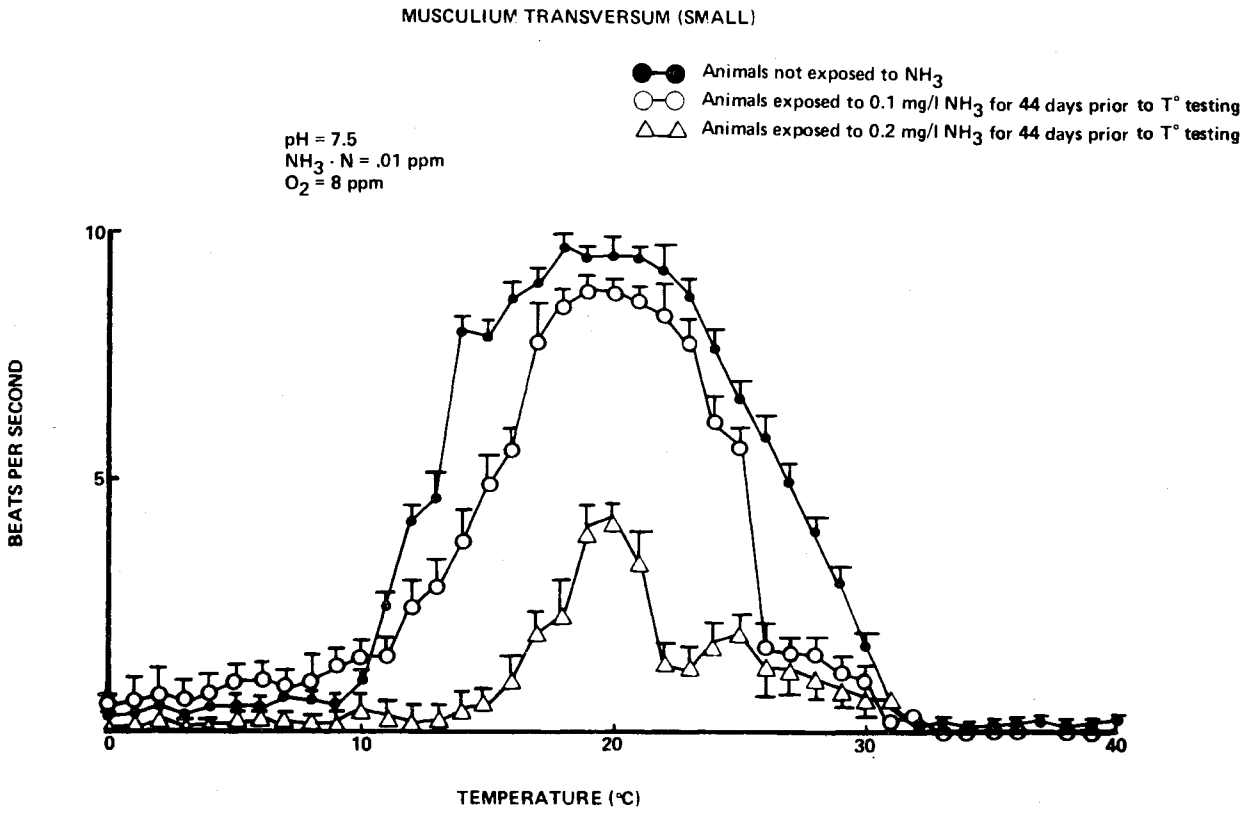



Figure 39. Previous exposure of clams to sublethal concentrations of un-ionized ammonia reduces the maximum ciliary response of their gills to temperature and also reduces the temperature range in which normal ciliary activity can be maintained.

Figure 39 shows that prior exposure to ammonia reduces both the maximum ciliary response to temperature and the temperature tolerance range. While previous exposure to 0.1 mg/l $NH_3$-N has a rather small, but detectable effect on the temperature response, previous exposure to 0.2 mg/l has a dramatic effect.

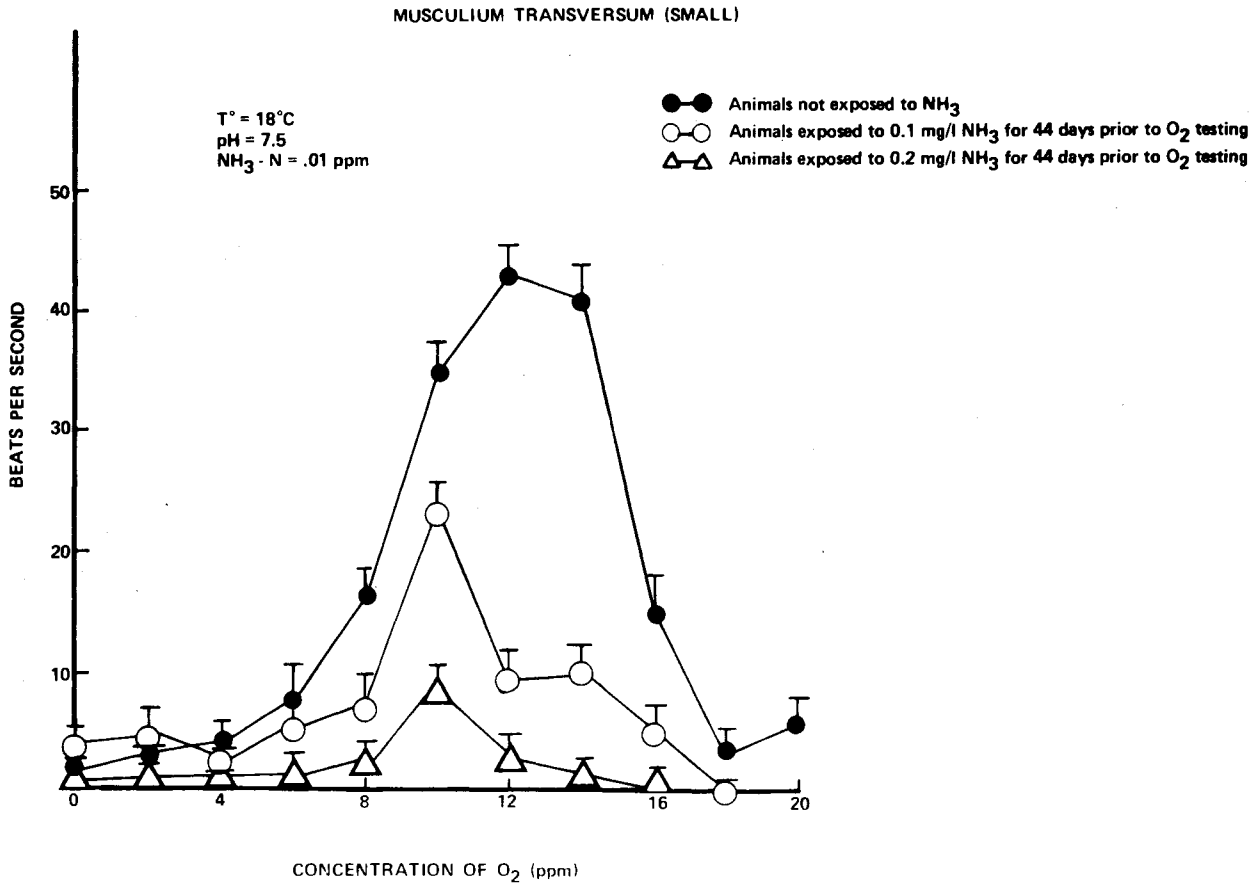



Figure 40. Previous exposure of fingernail clams to sublethal concentrations of un-ionized ammonia reduces the ability of the gills to increase their ciliary beating rate in response to increasing concentrations of oxygen.

Figure 40 shows that previous exposure of fingernail clams to sublethal concentrations of un-ionized ammonia reduces the ability of the gills to increase their ciliary beating rate in response to increasing concentrations of oxygen and Figure 41 shows that decreasing concentrations of oxygen have a greater inhibitory effect on the ammonia-exposed gills than on the gills not exposed to ammonia.

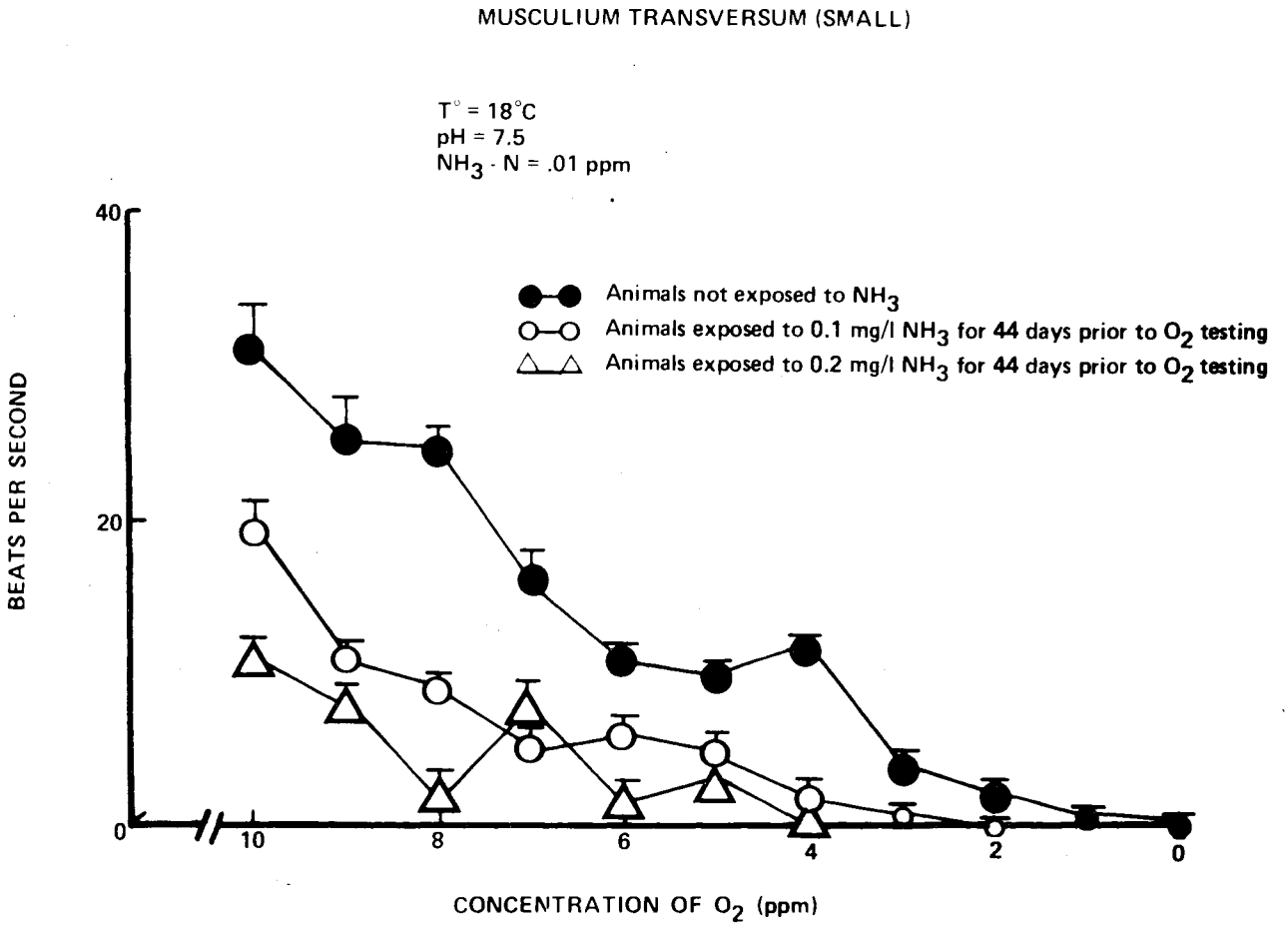



Figure 41. Low oxygen concentrations cause greater inhibition of ciliary beating rates on gills from clams previously exposed to sublethal concentrations of ammonia than on gills from clams not exposed to ammonia.

Relative Sensitivity of Fingernail Clams and Other Aquatic Organisms to Ammonia. The results of the chronic ammonia bioassay indicate that M. transversum is sensitive to un-ionized ammonia. Data from ammonia bioassay $NH_3 2$ demonstrated a MATC for survival between 0.35 and 0.59 mg/1 un-ionized $NH_3$-N. Data from ammonia bioassay $NH_3 3$ demonstrated a MATC for survival between 0.34 and 0.60 mg/1 un-ionized $NH_3$-N and a MATC for growth between 0.20-0.34 mg/1 un-ionized $NH_3$-N. The sensitivity of M. transversum to ammonia is similar to the sensitivity of rainbow trout with concentrations

between 0.4 and 1.8 mg/l un-ionized ammonia reported to be lethal to rainbow trout depending on the free $CO_2$ and dissolved oxygen concentrations (Merkens and Downing, 1957, Lloyd and Herbert, 1960). The bluegill sunfish, Lepomis macrochirus, a warm-water species, is more tolerant of ammonia than M. transversum with the lethal threshold reported as 1.65 mg/l un-ionized $NH_3$ (1.36 mg/l $NH_3$-N) (Lubinski, Sparks, and Jahn, 1974). The only usable ammonia data for an invertebrate species that could be found in the literature was a 2-day LC50 of 0.66 mg/l un-ionized $NH_3$ (0.54 mg/l $NH_3$-N) for Daphnia magna (European Inland Fisheries Advisory Commission, 1970).

Although no data are available yet, it is probable that the reproduction of the fingernail clam could be affected at un-ionized ammonia concentrations below 0.20-0.34 mg/l ($NH_3$-N). The ciliary response of gills from large clams is markedly affected at concentrations of .03 mg/l $NH_3$-N. The ciliary beating response of adult fingernail clams is slightly more sensitive to un-ionized ammonia than that of the Asiatic clam (Corbicula manilensis), a freshwater unionid mussel (Elliptio complanata), and a marine mussel (Mytilus edulis), as can be seen by comparing Figures 29 and 30. Chronic exposure of clams to sublethal concentrations of ammonia lowers the tolerance of the gills to a variety of stresses, including additional exposure to ammonia (see discussion in the above section).

Relationship between Ammonia Levels in the Illinois and Mississippi Rivers and Ammonia Levels Which Affected Fingernail Clams in Laboratory Experiments. The total $NH_3$-N concentrations in the Illinois and Mississippi Rivers in 1975 (Tables 2-4) were converted to un-ionized ammonia concentrations ($NH_3$-N, mg/l) using tables provided by Thurston et al. (1974: 9-15), and mean or median pH and temperature values reported by the Illinois Environmental Protection Agency

(1975, Volume 4: 425; Volume 2: 92-93, 103, 104). The median pH and temperature were used to convert the median ammonia concentrations (Table 2), while the mean pH and temperature were used to convert both the mean and the maximum ammonia concentrations (Tables 3 and 4). The un-ionized ammonia nitrogen concentrations ($NH_3$-N, mg/l) occurring in 1975 are given below:

| | Mississippi River | | Illinois River | | |
|---|---|---|---|---|---|
| | Rt. 9 Bridge Ft. Madison, IA Mile 383.9 | Rt. 150 Bridge Peoria,IL Mile 165.8 | Lock and Dam Creve Coeur, IL Mile 157.7 | Rt. 9 Bridge Pekin, IL Mile 152.9 | Rt. 97 Bridge Havana, IL Mile 119.5 |
| Median | .005 | .021 | .012 | .031 | .012 |
| Mean | .010 | .027 | .021 | .027 | .018 |
| Maximum | .058 | .125 | .074 | .098 | .048 |

Although it would be better to convert the total ammonia concentrations to un-ionized ammonia concentrations using the pH and temperatures actually occurring at the time the ammonia samples were taken, rather than mean or median pH and temperatures, the results do indicate that the concentrations of un-ionized ammonia in the Illinois River in 1975 were approximately twice the concentrations in the Keokuk Pool, Mississippi River. Moreover, the mean and median concentrations at the four Illinois River stations where fingernail clams have died out were close to the value of .03 mg/l which caused a 50% reduction in the ciliary beating rate of gills from large fingernail clams.

Recently, Thompson and Sparks (1977) reported an alarming decrease in the fingernail clam populations in the Keokuk Pool, Mississippi River (Figure 42).

Not only did the number of clams decline, but the growth of the survivors was reduced (Figure 43). In 1976, the maximum shell length was only

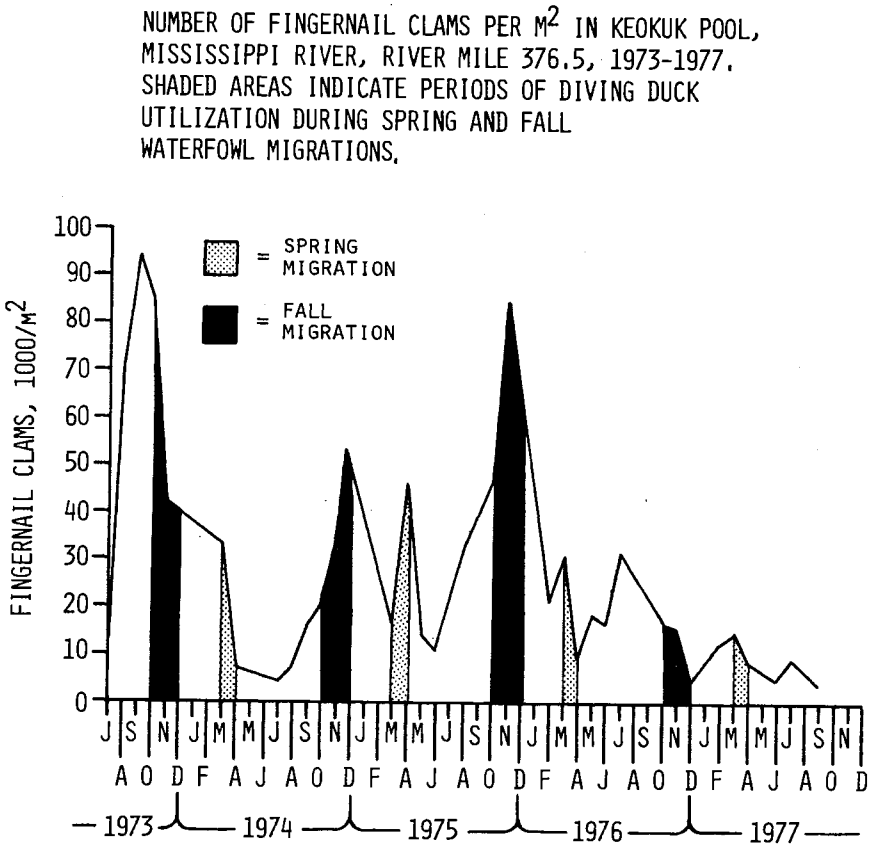



Figure 42. The peak number of fingernail clams in bottom samples from Keokuk Pool, Mississippi River declined by 90% in 1976-1977.

8.3 mm as compared to 12.4 mm in both 1974 and 1975. Fingernail clams begin to reproduce when they reach about 5 mm in shell length (Gale, 1969). In 1974 and 1975, the reproductive population numbered 5,000-6,000 clams per square meter. In 1976, the reproductive population was reduced to less than 1,000 per square meter.

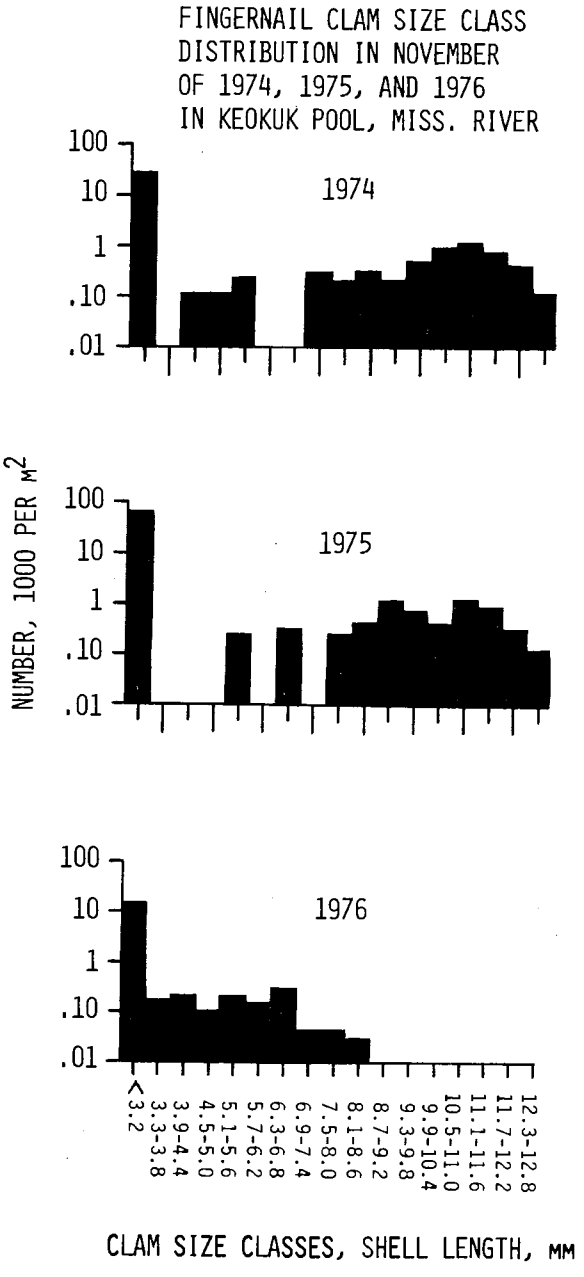



Figure 43. The growth of fingernail clams in the Keokuk Pool, Mississippi River, was reduced in 1976. In 1976 the maximum individual shell length was only 8.3 as compared to 12.4 in both 1974 and 1975.

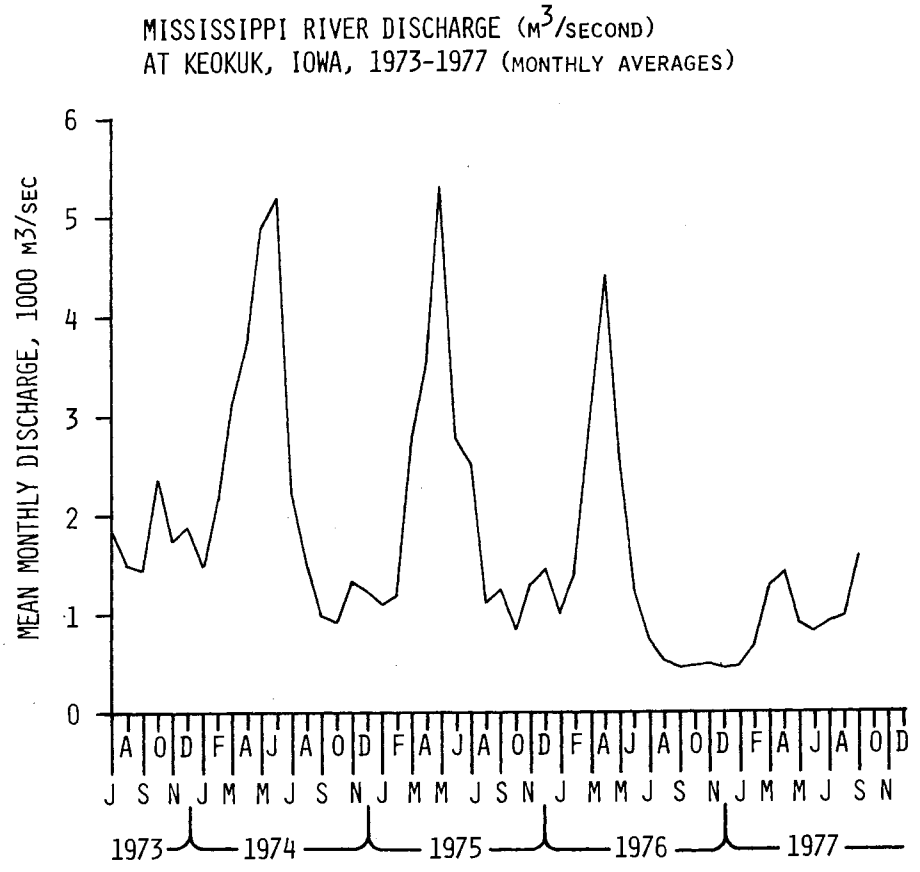



Figure 44. Mississippi River discharge at Keokuk, Iowa, showing the effects of the 1976-1977 drought.

Both the low clam population and reduced growth Thompson and Sparks observed in 1976 and 1977 appeared to be related to an extremely low river discharge as a consequence of a drought in the upper Mississippi Basin. In Figure 44, the Mississippi discharge at Keokuk, Iowa is graphed. The graph shows the usual cycle of spring highs and summer lows. However, the low discharge period in 1976 and spring discharge in 1977 were much reduced from those of previous years. During this low discharge period, Thompson and Sparks found effects on certain water parameters such as lower dissolved oxygen concentrations, dissolved oxygen stratification, increased water clarity, and the elevation in concentrations of certain materials such as un-ionized ammonia ($NH_3$-N).

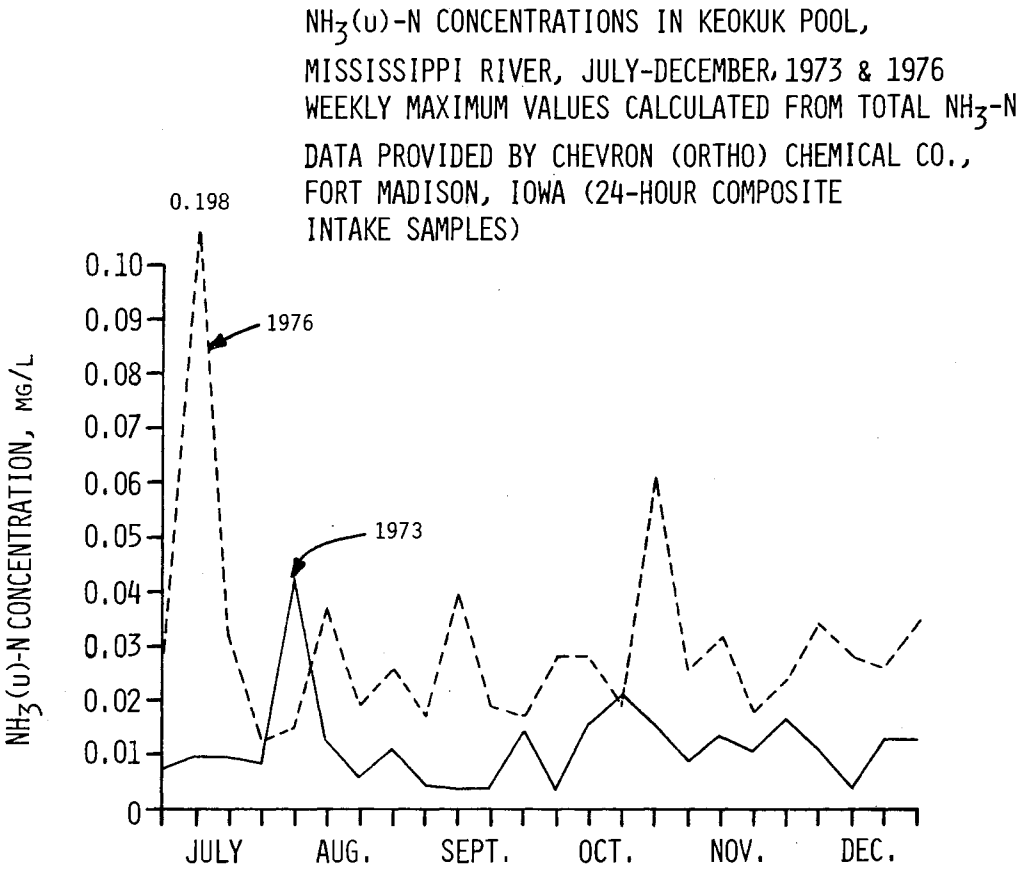



Figure 45. The concentration of un-ionized ammonia ($NH_3$-N) in the Keokuk Pool, Mississippi River, was greater in 1976 than in 1973.

Figure 45 compares the weekly maximum concentrations for un-ionized ammonia for the low discharge period July-December in 1973 and 1976. We found that during the 1976 period that these values were elevated well above the 1973 concentrations. The highest value of 0.198 mg/l $NH_3$-N is very near the level of 0.34 mg/l which affected the growth of fingernail clams in our laboratory bioassays after two weeks of continuous exposure, and the average values of .02 to .03 mg/l were close to, or within the range which caused a 50% reduction in the ciliary beating rate of gills from large clams.

Our results with the gill preparations show that they become more sensitive to low oxygen levels and ammonia, following chronic exposure to

un-ionized ammonia concentrations of 0.10 or 0.20 mg/l. Since the dissolved oxygen levels on the bottom in Keokuk Pool were reduced during the drought, and toxicants, such as heavy metals, were presumably not diluted as much in 1976-1977 as in previous years, one can speculate that the combined action of all these factors was sufficient to stress the fingernail clams in Keokuk Pool. Our results also showed that as the fingernail clams grow, they become less tolerant of extremes in environmental factors such as temperature and low dissolved oxygen, and less tolerant of toxicants.

We speculate that in 1976-1977, the fingernail clams in Keokuk Pool grew up to the point where their ciliary activity was impaired by environmental factors. At that point, their ability to feed and respire would be impaired, and they would grow slowly, if at all. Since the clams must reach a size of approximately 5 mm to reproduce, and the number of young produced increases as the size of the parent increases, the severe reduction in growth also caused a severe reduction in reproduction, hence reduced numbers of clams in 1976-1977. This interpretation could be confirmed by reproducing the 1976-1977 conditions in the laboratory, and observing the effect on clam growth and reproduction.

Response of Clams to Suspended Particles

Table 6 shows that the particle transport rate of gills from large clams was more sensitive to suspended particles than the transport rate of gills from small clams. Table 6 also shows that in solutions containing equal numbers of particles per liter, sharp silica particles impaired the transport rate more than rounded illite clay particles. The transport rate was reduced more at low concentrations of oxygen when the particle concentration was increased. The particle concentrations (number

Table 6 . Effect of Oxygen Tension and Suspended Particles on the Average Rate of Transport of Particles on the Gills of Musculium transversum.[a]

| Particles Per Liter | Average Rate of Transport (μm/sec.$^{-1}$) Small Clams | | | | | Large Clams | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2 ppm | 4 ppm | 6 ppm | 8 ppm | 10 ppm | 2 ppm | 4 ppm | 6 ppm | 8 ppm | 10 ppm |
| | Illite Clay Particles (Mean size = 2.8 μm, standard deviation = 0.9 μm) | | | | | | | | | |
| $10^1$ (control) | 155.3[b] ±12.2 | 150.2 | 168.9 ±11.8 | 169.7 ±10.2 | 165.4 ± 2.8 | 10.1 ±0.3 | 28.1 ±0.7 | 40.0 ±0.9 | 80.1 ±0.5 | 90.3 ±6.9 |
| $5x10^1$ | 30.2 ±13.8 | 28.7 ±1.2 | 89.2 ±10.2 | 180.0 ±10.3 | 151.2 ±10.2 | 8.3 ±0.3 | 11.3 ±0.1 | 30.1 ±0.4 | 60.2 ±0.1 | 80.5 ±7.8 |
| $10^2$ | 12.2 ±12.8 | 15.2 ±0.8 | 70.2 ±9.7 | 153.4 ± 5.8 | 161.3 ± 1.3 | 3.0 ±0.1 | 3.3 ±0.1 | 11.3 ±0.2 | 48.2 ±0.2 | 72.8 ±3.2 |
| $10^3$ | 10.2 ±0.8 | 11.2 ±0.1 | 50.0 ±2.8 | 138.3 ± 3.2 | 141.3 ±21.2 | 0 | 0 | 7.3 ±0.5 | 31.4 ±0.6 | 61.3 ±1.9 |
| $10^4$ | 10.3 ±0.1 | 10.3 ±0.3 | 42.0 ±0.8 | 110.3 ± 3.9 | 128.4 ± 8.2 | 0 | 0 | 3.3 ±0.3 | 15.3 ±0.9 | 52.9 ±1.2 |
| $10^5$ | 10.2 ±0.1 | 7.3 ±0.6 | 43.3 ±0.8 | 90.8 ±3.2 | 115.1 ±12.2 | 0 | 0 | 1.6 ±0.2 | 12.3 ±0.3 | 20.4 ±0.5 |
| $10^6$ | 10.2 ±0.1 | 8.5 ±0.6 | 38.2 ±0.2 | 60.2 ±1.9 | 110.1 ± 8.2 | 0 | 0 | 0 | 10.7 ±0.1 | 21.3 ±0.7 |
| $10^7$ | 9.8 ±0.2 | 10.8 ±0.7 | 27.5 ±1.6 | 31.2 ±3.9 | 61.2 ±9.8 | 0 | 0 | 0 | 3.2 ±0.1 | 19.8 ±0.2 |
| | Silica Flour Particles (Mean size = 3.3 μm, standard deviation = 0.4 μm) | | | | | | | | | |
| $10^1$ (control) | 32.3 ±2.3 | 50.2 ±1.8 | 149.4 ±17.7 | 143.7 ± 3.2 | 155.8 ± 9.6 | 9.5 ±0.8 | 15.5 ±1.2 | 30.2 ±3.9 | 39.6 ±1.8 | 55.2 ±10.2 |
| $5x10^1$ | 17.2 ±0.8 | 28.1 ±2.3 | 32.3 ±2.8 | 58.7 ±8.9 | 109.2 ± 7.8 | 0 | 2.3 ±0.8 | 15.3 ±3.2 | 18.7 ±1.8 | 25.2 ±8.9 |
| $10^2$ | 2.1 ±0.6 | 15.2 ±3.5 | 21.8 ±3.5 | 42.6 ±8.6 | 92.6 ±6.9 | 0 | 0 | 0 | 11.2 ±1.9 | 15.2 ±6.3 |
| $10^3$ | 0 | 0 | 9.3 ±1.7 | 31.2 ±2.5 | 91.9 ±9.3 | 0 | 0 | 0 | 8.5 ±1.2 | 6.2 ±0.8 |
| $10^4$ | 0 | 0 | 6.5 ±0.8 | 12.8 ±0.8 | 82.7 ±11.2 | 0 | 0 | 0 | 6.3 ±0.8 | 1.2 ±0.1 |
| $10^5$ | 0 | 0 | 3.2 ±0.2 | 2.3 ±0.2 | 61.2 ±6.3 | 0 | 0 | 0 | 0 | 1.8 ±0.1 |
| $10^6$ | 0 | 0 | 3.1 ±0.2 | 1.8 ±0.1 | 42.7 ±2.5 | 0 | 0 | 0 | 0 | 0 |
| $10^7$ | 0 | 0 | 0 | 0 | 35.8 ±1.9 | 0 | 0 | 0 | 0 | 0 |

[a]Each point represents an average of 14 gills and 12 readings per gill, or a total of 168 observations.

[b]Mean ± standard deviation.

per liter) which caused at least a 50% reduction in the particle transport rates of gills from large and small clams at various oxygen concentrations, are given below. The weight of suspended matter per liter, in mg/1, is also given. Where one concentration caused less than a 50% reduction and the next higher concentration caused more than a 50% reduction, a range is given to indicate that the concentration having a 50% effect lay between the two concentrations which were tested. The numbers of particles per liter were converted to milligrams per liter using the following relationships: $2.3 \times 10^6$ illite particles per liter weighed 233.8 mg/1, and $3.6 \times 10^6$ silica particles per liter weighed 138.2 mg/1.

Concentrations of Suspended Particles, or Concentration Ranges, Causing At Least a 50% Reduction in Particle Transport Rates of Clam Gills

| D.O., mg/1 | Small Clams | Large Clams |
|---|---|---|
| | Illite Clay Particles | |
| 10 | $10^6 - 10^7$ particles/1<br>102 - 1,016 mg/1 | $10^4$ particles/1<br>1.02 mg/1 |
| 8 | $10^5$ particles/1<br>10.2 mg/1 | $10^2 - 10^3$ particles/1<br>.01-.10 mg/1 |
| 6 | $5 \times 10^1$ particles/1<br>.005 mg/1 | $5 \times 10^1 - 10^2$ particles/1<br>.005 - .01 mg/1 |
| 4 | $10^1 - 5 \times 10^1$ particles/1<br>.001 - .005 mg/1 | $5 \times 10^1$ particles/1<br>.005 mg/1 |
| 2 | $10^1 - 5 \times 10^1$ particles/1<br>.001 - .005 mg/1 | $5 \times 10^1 - 10^2$ particles/1<br>.005 - .01 mg/1 |
| | Silica Flour Particles | |
| 10 | $10^4$ particles/1<br>.384 mg/1 | $5 \times 10^1$ particles/1<br>.002 mg/1 |
| 8 | $5 \times 10^1$ particles/1<br>.002 mg/1 | $5 \times 10^1$ particles/1<br>.002 mg/1 |
| 6 | $10^1 - 5 \times 10^1$ particles/1<br>.0004 - .002 mg/1 | $5 \times 10^1$ particles/1<br>.002 mg/1 |
| 4 | $5 \times 10^1$ particles/1<br>.002 mg/1 | $10^1 - 5 \times 10^1$ particles/1<br>.0004 - .002 mg/1 |
| 2 | $5 \times 10^1$ particles/1<br>.0004 mg/1 | $10^1 - 5 \times 10^1$ particles/1<br>.0004 - .002 mg/1 |

Total suspended solids (TSS) was measured by the Illinois Environmental Protection Agency just once in 1975 at one of the four sampling stations in the reach of the Illinois River where the fingernail clams died out, and TSS was not measured at all at the station on the Keokuk Pool, Mississippi River. The one TSS value for the Illinois River at the U.S. 150 bridge at Peoria was 700 mg/1. The suspended sediment in the Illinois River probably is comprised largely of illite clay, and 700 mg/1 thus would be sufficient to reduce the particle transport rate of gills from small clams by at least 50%, even if the oxygen levels in the water were at saturation or above. If the oxygen levels in the water were lower, the reduction in particle transport rates should be even greater, and gills from large clams would suffer even greater transport inhibition than gills from small clams.

Response of Clams to Raw Illinois River Water

Response of Gill Preparations. Figure 46 shows that the beating of cilia on the gills of small fingernail clams was almost completely inhibited when the gills were exposed to water taken from the Illinois River on October 5, 1977. Normal ciliary activity was maintained when clam gills were exposed to water taken from a shallow, sand-point well located 100 feet from the Illinois River. Partial inhibition of the cilia occurred when the river water was diluted with the well water. No additional effect on the cilia was observed after the diluted water had been stored in a metal reservoir, coated with aluminum paint, for several days.

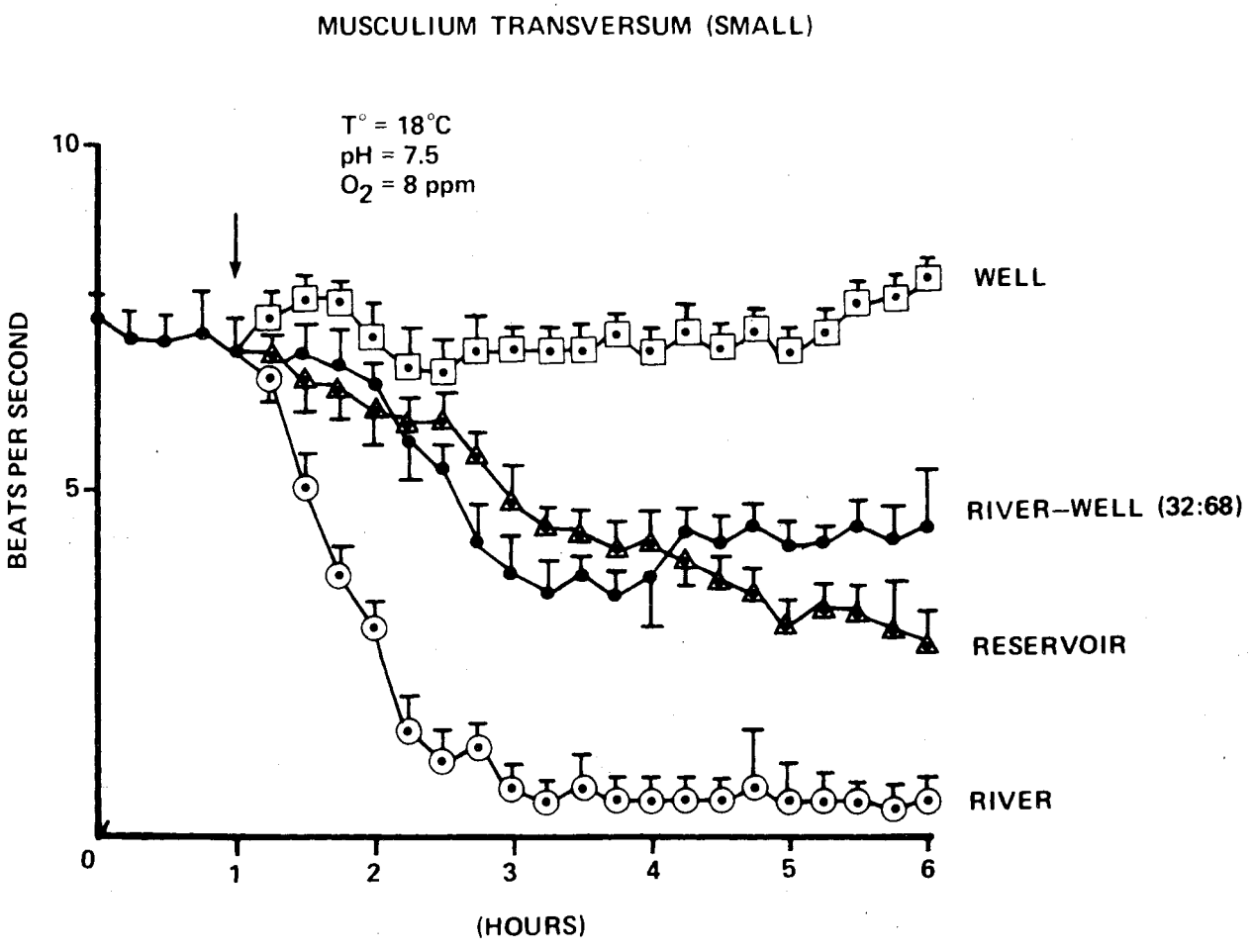



Figure 46. The ciliary activity of gills from small fingernail clams was almost completely inhibited when the gills were exposed to water taken from the Illinois River. Normal ciliary activity was maintained in well water. An intermediate response occurred in river water diluted with well water and no additional effect on the gills was obtained after the diluted water was stored several days in an aluminum-painted steel reservoir.

Figure 47. The white arrows show the curved shells which developed when the clams were exposed to Illinois River water which had been contaminated with metals. The shells were curved back to such an extent that the clam could not completely close them. All the clams with deformed shells eventually died.

Chronic Response of Clams to Raw Illinois River Water. On September 7, 1977 fingernail clams were collected from the Keokuk Pool and delivered to the laboratory at Havana, where they were acclimated to well water for one month. Starting October 12, 1977 the clams were exposed to continuous flows of raw Illinois River water, well water, or to river water diluted with well water. During the next four weeks, the clams developed shell deformities (Figure 47) and died without reproducing, with the greatest mortality and highest incidence of shell deformities appearing in test chambers containing the most river water.

Unfortunately, subsequent analysis of the river water revealed that the water had become contaminated with metals while it was being pumped from the

river into the test chambers. The metal content of the water in the river, and of the same batch of water after it had been pumped through the reservoirs and diluters, is presented below, for comparison.

| | Concentration in river water (mg/l) | Concentration in same river water, after being delivered to test chambers (mg/l) |
|---|---|---|
| potassium | 8.3 | 6.8 |
| aluminum | 2.1 | 2.3 |
| calcium | 58 | 58 |
| cadmium | <0.01 | 0.42 |
| chromium | <0.01 | 0.05 |
| cobalt | <0.01 | 0.09 |
| copper | 0.23 | 0.52 |
| iron | 3.5 | 2.2 |
| magnesium | 19 | 20 |
| manganese | 0.12 | 0.17 |
| lead | 0.33 | 2.7 |
| zinc | 0.14 | 0.62 |

It is apparent that the shell deformities and mortalities cannot be attributed to the Illinois River water, and that this experiment should be repeated after the metal parts in the intake system are replaced with plastic fittings.

Analysis of Shells from Deformed Clams. The deformed shells from clams which had been exposed to raw Illinois River water were sent to Dr. Judith Murphy, Director of the Center for Electron Microscopy at Southern Illinois University for elemental analysis using an X-ray microprobe. As expected, the principal component of the shell is calcium in the shell layers which were laid down when the clams were still in the Mississippi River. After the clams were introduced to the contaminated river water, however, the

proportion of calcium dropped and silicon became the predominant element. The phosphorus and sulfur levels were also somewhat elevated in the deformed areas. The microprobe technique does not measure the absolute amount of each element present, but the relative amount. At any rate, it is clear that the calcium/silicon ratio in the normal part of these shells is almost the reverse of that in the abnormal part. It appears that some contaminant in the river water interfered with normal calcium metabolism during shell formation.

## SUMMARY

1. Two methods for rapidly assessing water quality effects on clams were used in this study. One method, which measures particle transport rate across the gills of clams, had been used previously in basic research on the function of the clam gill. The second method, which measures the beating rate of the lateral cilia on the gill, had been used to test the effects of drugs on ciliary activity. New methods and equipment for measuring the ciliary activity of clams as small as 2 mm in shell length were developed as part of this research.

2. The research also developed culture techniques and chronic and acute bioassay methods for use with the fingernail clam, Musculium transversum, which died out in the Illinois River in the mid-1950's and which underwent a population decline in the Keokuk Pool, Mississippi River, in 1976-77. Following the die-off of fingernail clams, the number of diving ducks utilizing the Illinois valley declined from an average of 1½ million to 160,000, and the abundance and average size of commercially important species of fish declined. Results of both the rapid testing methods and the chronic and acute bioassays showed that the fingernail clams become more sensitive to toxicants and less tolerant of extremes in environmental factors as they grow older and larger. This means that it is the older, reproductive portion of the population which is most sensitive to environmental factors.

3. The ciliary beating rates of gills from two sphaeriacean clams (Musculium transversum and Corbicula manilensis) and the unrelated intertidal mussel (Mytilus edulis) were noticeably inhibited by light within 1 to 2½ hours

of exposure. Maximum inhibition occurred after 2 to 6 hours exposure. When the gill preparations were returned to darkness, the ciliary beating rates returned to normal after 2 to 4 hours, with the exception of large Musculium transversum, whose ciliary beating rates had not recovered to normal by the time the experiment was ended after 4 hours exposure to darkness. Perhaps the inhibition of cilia by light is a protective response, should the clam be exposed to falling water levels or to the air. The shell of adult fingernail clams is translucent, and the shells of small clams are transparent.

4. Gill preparations from the intertidal mussel, Mytilus edulis, maintained their ciliary beating rates over a broader temperature range than gill preparations from fingernail clams or Asiatic clams. Small fingernail clams had a broader temperature range than large ones, and the Asiatic clam appeared to have the narrowest temperature tolerance, at least as measured by the ciliary beating response. Additional experiments would have to be performed to determine the acclimation range of these three species of clams, and the relationship between the ciliary beating response and acclimation temperature. Fingernail clams begin to grow rapidly when water temperatures in the Keokuk Pool, Mississippi River, rise above 11-13 C. The ciliary beating rate of the fingernail clam substantially increased above 14-15 C, so the field results appear to corroborate the laboratory findings. The laboratory results also suggest that the ciliary function of fingernail clams would be drastically reduced if water temperatures increased above 30 C.

5. The higher the concentration of dissolved oxygen in the water perfusing the gill preparations, the greater the ciliary beating rate. The ciliary beating rate of fingernail clams rapidly declined to zero when the dissolved oxygen concentration was reduced to 2 ppm. It is unlikely that dissolved oxygen concentrations of 2 ppm or lower would immediately kill fingernail clams

in nature, as the clams can probably switch from aerobic to anaerobic metabolism when their shells are closed. The clams have oxygen-sensing organs, the palps, located on the gills. When the palps were removed, the gill preparations behaved as though they were in water containing little dissolved oxygen, even though oxygen levels in the water were maintained at 10 ppm.

6. The highest concentrations of sodium nitrate and sodium sulfate tested had no effect on the ciliary beating rates of gills from large or small clams. The maximum nitrate concentration we tested was equivalent to 18 mg/l as nitrogen, and the maximum sulfate concentration tested was 48 mg/l.

7. Fingernail clam gills were extremely sensitive to copper, lead, and zinc, and gills from large clams generally were several orders of magnitude more sensitive than gills from small clams. The following concentrations caused 90% reductions in the ciliary beating rates of gills from large clams: lead (2 µg/l), copper (.06-.6 µg/l), and zinc (.06-.65 mg/l). Several toxic metals, including lead and copper, occur at higher concentrations in the Illinois River than in the Mississippi. In view of the extreme sensitivity of the clam gills to copper, lead, and zinc, it appears that metals in the Illinois River could be a significant stress on adult fingernail clams. This tentative conclusion, which is based on tests with the sensitive gill preparations, should be verified with additional bioassays using intact fingernail clams.

8. There were significant differences in the responses of gills from large (6-11 mm) and small (2-5 mm) clams to: (a) removal and subsequent addition of potassium, (b) variation of maintenance dosage of potassium in the solution which bathed the gills, and (c) lag period of response to a specific dose. Potassium levels required for maintenance of a basal ciliary beating rate were $10^{-3}$ M (39.1 mg/l) for small clams and $10^{-6}$ M (0.039 mg/l) for

large clams. Greater concentrations were cilioinhibitory. Lesser concentrations were generally cilioexcitatory, but concentrations less than $10^{-8}$ M (0.00039 mg/l) and $10^{-9}$ M (0.000039 mg/l) are insufficient to sustain basal rates in large and small clams, respectively. The intact clams were much less sensitive to potassium than the gill preparations. The maximum acceptable toxicant concentration (MATC) for long-term survival of fingernail clams lies between 195 and 275 mg/l potassium. Potassium concentrations below the lethal level actually stimulated the growth of fingernail clams, so potassium appears to have no sublethal effects that result in reduced growth. However, the effects of potassium on the reproduction of the clams was not determined in these experiments. Musculium transversum was more sensitive to potassium than fish, about as sensitive as several microcrustaceans, and less sensitive than three species of unionid clams. The highest potassium concentration in 25 water samples taken near the surface of the Illinois River in 1975 was 6 mg/l potassium, well below the lethal threshold. However, potassium concentrations as high as 250 mg/kg have been found in the sediments of the Illinois River, so it is possible that water associated with these sediments provides considerably higher potassium concentrations than water above the sediments. Additional research is needed in this area.

9. Clams exposed to potassium in water with a total hardness equal to 243 mg/l as $CaCO_3$, responded faster than clams tested in water with a total hardness equal to 314 mg/l as $CaCO_3$. The toxicity curve of the soft-water test approached a vertical asymptote at 400 hours, while the clams tested in the hard water did not show a lethal threshold even at 696 hours.

10. Clams exposed to potassium at a water temperature of 6.5 C died more slowly than those in water at a temperature of 16.7 C, but the lethal

threshold concentration of potassium was not changed.

11. Large fingernail clams were more sensitive to un-ionized ammonia than small fingernail clams, the Asiatic clam (Corbicula manilensis), a freshwater unionid mussel (Elliptio complanata), and the intertidal mussel (Mytilus edulis). An un-ionized ammonia concentration of .03 mg/l (as ammonia nitrogen, $NH_3$-N) caused a 50% reduction in the ciliary beating rate of gills from large clams, and a concentration of .05-.06 mg/l caused complete inhibition of the cilia. The sensitivity of the gill preparations to un-ionized ammonia increased as the oxygen content of the water decreased. Results of the chronic bioassay showed that the maximum acceptable toxicant concentration (MATC), based on mortality, lies between .34 and .60 mg/l $NH_3$-N, and the MATC based on growth lies between 0.20 and 0.34 mg/l $NH_3$-N.

12. Chronic exposure of clams to sublethal concentrations of ammonia lowers the tolerance of their gills to a variety of stresses, including additional exposure to ammonia. Previous exposure of clams to 0.2 mg/l $NH_3$-N for 44 days completely blocked the normal stimulatory response of the gills to potassium addition. In fact, the ciliary beating rate was slightly to markedly inhibited at all potassium concentrations tested. The gills of the clams were sensitized to ammonia by previous exposure to sublethal concentrations of ammonia. A 50% reduction in the ciliary beating rate occurred at an un-ionized ammonia concentration of .09 mg/l ($NH_3$-N) in control gills not previously exposed to ammonia, at .04 mg/l in gills previously exposed to 0.1 mg/l $NH_3$-N for 44 days, and at .01-.02 mg/l in gills previously exposed to 0.2 mg/l $NH_3$-N for 44 days. Prior exposure to sublethal concentrations of ammonia also reduced both the maximum ciliary response to temperature and the temperature tolerance range. Decreasing concentrations of oxygen had a greater inhibitory effect on ammonia-exposed gills than on gills not exposed to ammonia.

13. Concentrations of un-ionized ammonia in the Illinois River in 1975 were approximately twice the concentrations in the Keokuk Pool, Mississippi River. Moreover, the mean and median concentrations at the four Illinois River stations where fingernail clams died out were close to the value of .03 mg/l $NH_3$-N which caused a 50% reduction in the ciliary beating rate of gills from large fingernail clams.

14. There has been an alarming decrease in the fingernail clam populations in the Keokuk Pool, Mississippi River in 1976 and 1977. The growth of the surviving clams was also reduced. In 1976, the maximum shell length was only 8.3 mm as compared to 12.4 mm in both 1974 and 1975. Fingernail clams begin to reproduce when they are about 5 mm in shell length. In 1974 and 1975, the reproductive population numbered 5,000-6,000 clams per square meter. In 1976, the reproductive population was reduced to less than 1,000 per square meter.

15. Both the low clam population and reduced growth in Keokuk Pool in 1976 and 1977 appear to be related to an extremely low river discharge as a consequence of a drought in the upper Mississippi River basin. The following effects on water quality factors were observed during this low-discharge period: lowered dissolved oxygen concentrations, dissolved oxygen stratification, increased water clarity, and elevation in concentrations of certain materials such as un-ionized ammonia. The highest un-ionized ammonia value measured in Keokuk Pool in 1976 near Fort Madison, Iowa was 0.198 mg/l $NH_3$-N, which is very near the level of 0.20-0.34 mg/l which affects the growth of fingernail clams in our laboratory bioassays after two weeks of continuous exposure. The average values of .02-.03 mg/l $NH_3$-N in Keokuk Pool were close to, or within, the range which caused a 50% reduction in the ciliary beating rate of gills from large clams. Our laboratory results also showed that the gills

become more sensitive to low oxygen levels and ammonia, following chronic exposure to un-ionized ammonia concentrations of 0.10 or 0.20 mg/1 $NH_3$-N. Since the dissolved oxygen levels on the bottom in Keokuk Pool were reduced during the drought, and toxicants, such as heavy metals, were presumably not diluted as much in 1976-1977 as in previous years, we hypothesize tha t the combined action of all these factors was sufficient to stress the fingernail clams in Keokuk Pool. Our results also showed that as the fingernail clams grow, they become less tolerant of extremes in environmental factors such as temperature and low dissolved oxygen, and less tolerant of toxicants. We hypothesize that in 1976-1977, the fingernail clams in Keokuk Pool grew up to the point where their ciliary activity was impaired by environmental factors. At that point, their ability to feed and respire was impaired, and they grew slowly, if at all. Since the clams must reach a size of 5 mm to reproduce, and the number of young produced increases as the size of the parent increases, the severe reduction in growth also caused a severe reduction in reproduction, hence reduced numbers of clams in 1976-1977. This interpretation could be confirmed by reproducing the 1976-1977 water quality conditions in the laboratory, and observing the effects on clam growth and reproduction.

16. The particle transport rate of gills from large clams was more sensitive to suspended particles than the transport rate of gills from small clams. Sharp silica particles impaired the transport rate of the gills more than rounded illite clay particles. The transport rate was reduced at low concentrations of oxygen. At a constant oxygen concentration of 8 mg/1, a 50% reduction in the particle transport rate of gills from small clams was induced by exposure to $10^5$ illite clay particles per liter (10.2 mg/1) and $5 \times 10^1$ silica particles

per liter (.002 mg/1). At the same oxygen concentration, a 50% reduction in the particle transport rate of gills from large clams was induced by exposure to $10^2$-$10^3$ illite clay particles per liter (.01-.10 mg/1) and 5 x $10^1$ silica particles per liter (.002 mg/1).

17. The beating of cilia on the gills of small fingernail clams was almost completely inhibited when the gills were exposed to a sample of water taken from the Illinois River on October 5, 1977. Normal ciliary activity was maintained when gills were exposed to water from a shallow, sand-point well located 100 feet from the Illinois River. Partial inhibition of the cilia occurred when the river water was diluted with the well water.

18. Fingernail clams developed bizarre shell deformities and died without reproducing, when they were exposed for four weeks to water containing a mixture of metals at the following concentrations: 6.8 mg/1 potassium, 2.3 mg/1 aluminum, 58 mg/1 calcium, 0.42 mg/1 cadmium, 0.05 mg/1 chromium, 0.09 mg/1 cobalt, 0.52 mg/1 copper, 2.2 mg/1 iron, 20 mg/1 magnesium, 0.17 mg/1 manganese, 2.7 mg/1 lead, and 0.62 mg/1 zinc. Further experiments are needed to determine which of the above metals, or combination of metals, produced the shell deformities and mortalities. X-ray microprobe analysis showed that the principal component of the shells was calcium in the shell layers which were laid down when the clams were still in the Mississippi River. After the clams were introduced to the water containing the metals, however, the proportion of calcium in the shell dropped, and silicon became the predominant element. Phosphorus and sulfur levels were also somewhat elevated in the deformed areas. It appears that some of the above metals interfere with normal calcium metabolism during shell formation.

# RECOMMENDATIONS

1. Additional bioassays should be performed to determine whether intact fingernail clams are as sensitive to metals as the isolated gill preparations.

2. The effects of raw Illinois River water on intact fingernail clams should be determined. If the raw water affects the intact clams, additional bioassays should be run in which fingernail clams are exposed to river water treated to remove certain components, such as silt, unionized ammonia, and heavy metals. The results of this deletion bioassay would establish whether fingernail clams could survive in the Illinois River if certain factors were reduced or removed by waste treatment.

3. Fingernail clams should be exposed in the laboratory to water quality conditions which existed in the Keokuk Pool in 1976-1977, to determine whether such factors as elevated ammonia levels and lowered dissolved oxygen levels were responsible for the reduced growth and reproduction of clams in the Pool in 1976-1977.

4. The techniques and apparatus for measuring the ciliary beating response of gills from clams should be considered as a candidate method for rapidly assessing the toxicity of new chemicals, before they are released to the aquatic environment.

## RELATION OF THIS RESEARCH TO WATER RESOURCES PROBLEMS

Fingernail clams play an important role in the ecology of Midwestern waters and are sensitive to water quality changes. An unexplained die-off of fingernail clams occurred in the Illinois River in 1955, with dramatic ecological repercussions. Similar losses could occur in other waters (there was, in fact, a dramatic reduction in fingernail clam populations in the Keokuk Pool, Mississippi River, in 1976-1977), unless the reasons for the die-off can be determined and prevented. By developing information on the effects of water quality on fingernail clams, this research has contributed to the national objective of predicting ecologic change and improving water quality. The research has developed a new method for rapidly assessing water quality effects on types of organisms for which no standard method now exists. The rapid development of this type of information should be of interest to those who must plan and manage water resource systems for a variety of beneficial uses, including recreation and the production of fish and waterfowl. The method might also be used to test new chemicals before they enter the aquatic environment. The technique might also be used to test control agents for pest species, such as the introduced Asiatic clam, which enters and clogs condenser tubes of power plants.

Requests for information regarding both the methods and data developed in this project have been received from Donovan M. Oseid of the University of Minnesota Department of Entomology, Fisheries, and Wildlife, and from Walter Ginsburg, Chief Water Bacteriologist of the City of Chicago Water Purification Laboratory. There have been 9 requests for reprints of publications resulting from this research. In addition, project results have been used by the Division of Water Resources, Illinois Department of Transportation, the Illinois Department of Conservation, and the U.S. Fish and Wildlife Service in reviewing permits for development along the Keokuk Pool, Mississippi River.

## ACKNOWLEDGEMENTS

We are grateful to the Division of Fisheries, Illinois Department of Conservation, and to Mr. Hubert Bell, Supervisor of the Fisheries Building at Havana, for providing laboratory space and assisting with the construction of a pipeline to bring Illinois River water into the laboratory. The Supporting Services of the Illinois Natural History Survey, including Robert O. Ellis, Assistant for Operations, and Larry D. Gross and Jerry McNear, Operations Assistants, also helped install the pipeline at the laboratory. Dr. Kenneth E. Smith and Dr. Allison Brigham, Illinois Natural History Survey at Urbana, analyzed water samples from the Mississippi and Illinois Rivers and from the laboratory bioassays. Dr. Dean Wesley, Department of Agriculture at Western Illinois University, provided a flame photometer and instruction for some chemical analyses. Mr. Tony Petrovich, owner and manager of Tony's Plumbing and Heating, Inc., in Alpha, Illinois, provided instruction and parts for construction of diluters and plumbing in the bioassay laboratories at Havana. Mr. Carl M. Thompson and Mr. Michael J. Sandusky provided much-needed assistance in both the laboratory and field. Mr. Harold Henderson helped count and measure fingernail clams during the chronic bioassays, and typed and edited the final report. Dr. Judith Murphy, Director of the Center for Electron Microscopy at Southern Illinois University, Carbondale, provided an elemental analysis of deformed clam shells, using electron microprobe techniques. We are grateful to Mr. A.A. Fitzsimmons and Mr. John L. Maier of Chevron Chemical Company, Fort Madison, Iowa, for supplying us with water quality data from their intake on Keokuk Pool. Finally, we are grateful to Dr. Glenn Stout, Director of the Water Resources Center at the University of Illinois, for helpful advice in the preparation of the initial research proposal, and for encouragement and understanding during the project and during preparation of the final report.

The work upon which this publication is based was supported by funds provided by the U.S. Department of the Interior as authorized under the Water Resources Research Act of 1965, P.L. 88-379.

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APPENDIX A. TEST CONDITIONS AND RESULTS OF ACUTE AND CHRONIC BIOASSAYS.

Table 7. Test Conditions for Adult Test A1.

| Test Chamber | Temperature (C) Mean | Range | pH [1] | Dissolved Oxygen (mg/l) Mean | Range | Total Alkalinity [1] (mg/l) as $CaCO_3$) | Potassium (mg/l) Mean | Range |
|---|---|---|---|---|---|---|---|---|
| 1 | 16.7 | 16.0-17.0 | 8.50 | 9.3 | 8.8-9.7 | 161 | 581.0 | 562.0-600.0 |
| 2 | 16.7 | 16.0-17.0 | 8.50 | 9.3 | 8.5-9.7 | 161 | 316.5 | 288.0-345.0 |
| 3 | 16.7 | 16.0-17.0 | 8.50 | 9.3 | 8.6-9.7 | 161 | 192.0 | 181.0-203.0 |
| 4 | 16.7 | 16.0-17.0 | 8.50 | 9.3 | 8.6-9.6 | 161 | 106.3 | 102.5-110.0 |
| 5 | 16.7 | 16.0-17.0 | 8.50 | 9.3 | 8.8-9.7 | 161 | 57.3 | 48.0- 66.5 |
| 6 | 16.7 | 16.0-17.0 | 8.50 | 9.3 | 8.8-9.7 | 161 | 37.0 | 34.0- 40.0 |
| 7 (control) | 16.7 | 16.0-17.0 | 8.50 | 9.3 | 8.9-9.8 | 161 | 9.5 | 7.3- 11.7 |

[1] Measured only once during the bioassay.

Table 8. Results of Test A1.

| Mean Potassium Concentration (mg/l) | Percent Dead at Hr 48 | Hr 72 | Hr 96 | Hr 120 |
|---|---|---|---|---|
| 581.0 | 55.6 | 85.7 | 100.0 | 100.0 |
| 316.5 | 27.2 | 60.0 | 80.0 | 90.0 |
| 192.0 | 12.5 | 37.5 | 50.0 | 50.0 |
| 106.3 | 11.1 | 11.1 | 22.2 | 22.2 |
| 57.3 | 0 | 10.0 | 10.0 | 10.0 |
| 37.0 | 0 | 10.0 | 10.0 | 10.0 |
| 9.5 (control) | 0 | 0 | 0 | 0 |
| LC50 | 518 | 255 | 185 | 168 |
| 95 percent confidence limits | 310-865 | 175-372 | 128-268 | 119-237 |
| slope function | 2.66 | 2.42 | 2.06 | 1.94 |

Table 9. Test Conditions for Adult Test A2.

| Test Chamber | Temperature (C) Mean | Range | pH Mean | Range | Dissolved Oxygen (mg/l) Mean | Range | Total Alkalinity (mg/l as CaCO3) Mean | Range | Potassium (mg/l) Mean | Range |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 17.0 | 16.3-17.3 | 8.22 | 8.18-8.28 | 9.3 | 9.1-9.4 | 154 | 152-156 | 2036.0 | 1956.0-2116.0 |
| 2 | 17.0 | 16.1-17.3 | 8.23 | 8.20-8.28 | 9.3 | 9.2-9.4 | 155 | 154-156 | 1139.0 | 1102.0-1176.0 |
| 3 | 16.8 | 15.9-17.1 | 8.27 | 8.25-8.30 | 9.3 | 9.2-9.4 | 158 | 156-160 | 606.0 | 599.0- 613.0 |
| 4 | 16.7 | 15.7-17.0 | 8.24 | 8.18-8.28 | 9.2 | 9.0-9.4 | 154 | 152-156 | 356.0 | 349.0- 360.0 |
| 5 | 16.9 | 15.9-17.3 | 8.25 | 8.18-8.32 | 9.3 | 9.1-9.4 | 154 | 150-160 | 190.3 | 177.8- 211.2 |
| 6 | 17.0 | 16.0-17.4 | 8.26 | 8.12-8.41 | 9.3 | 9.2-9.4 | 155 | 153-156 | 108.1 | 106.0- 109.6 |
| 7 | 16.9 | 15.9-17.3 | 8.22 | 8.15-8.38 | 9.2 | 9.0-9.4 | 155 | 152-156 | 64.1 | 63.3- 64.7 |
| 8 (control) | 17.0 | 16.0-17.5 | 8.23 | 8.13-8.28 | 9.2 | 9.0-9.4 | 152 | 152-153 | 2.6 | 2.0- 3.0 |

Table 10. Results of Test A2.

| Mean Potassium Concentration (mg/l) | Percent Dead at Hr 48 | Hr 72 | Hr 96 | Hr 144 | Hr 192 | Hr 240 | Hr 312 |
|---|---|---|---|---|---|---|---|
| 2036.0 | 95 (94.7) [1] | 100 | 100 | 100 | 100 | 100 | 100 |
| 1139.0 | 75 (73.7) | 100 | 100 | 100 | 100 | 100 | 100 |
| 606.0 | 25 (21.0) | 100 | 100 | 100 | 100 | 100 | 100 |
| 356.0 | 5 (0) | 45 (42.1) | 80 (78.9) | 95 (94.7) | 100 | 100 | 100 |
| 190.3 | 0 (0) | 10 ( 5.3) | 15 (10.5) | 25 (21.1) | 40 (36.8) | 50 (47.4) | 50 (47.4) |
| 108.1 | 5 (0) | 5 (0) | 5 (0) | 15 (10.5) | 15 (10.5) | 15 (10.5) | 15 (10.5) |
| 64.1 | 5 (0) | 10 ( 5.3) | 10 ( 5.3) | 10 ( 5.3) | 10 ( 5.3) | 10 ( 5.3) | 10 ( 5.3) |
| 2.6 (control) | 5 (0) | 5 (0) | 5 (0) | 5 (0) | 5 (0) | 5 (0) | 5 (0) |
| LC50 | 880 | 370 | 280 | 228 | 212 | 200 | 200 |
| 95 percent confidence limits | 707-1094 | 293-467 | 229-342 | 185-281 | 167-270 | 156-257 | 156-257 |
| slope function | 1.65 | 1.46 | 1.38 | 1.40 | 1.48 | 1.78 | 1.78 |

[1] Numbers in parentheses indicate values corrected for control mortality.

Table 11. Test Conditions for Juvenile Test J1.

| Test Chamber | Temperature (C) Mean | Range | pH Mean | Range | Dissolved Oxygen (mg/l) Mean | Range | Total Alkalinity[1] (mg/l as $CaCO_3$) | Potassium (mg/l) Mean | Range |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 17.0 | 16.9-17.2 | 8.47 | 8.40-8.50 | 9.2 | 9.0-9.3 | 161 | 3532.0 | 3300-3680 |
| 2 | 17.0 | 16.9-17.2 | 8.49 | 8.40-8.55 | 9.2 | 9.1-9.2 | 161 | 2103.0 | 1920-2220 |
| 3 | 17.0 | 16.9-17.2 | 8.49 | 8.45-8.55 | 9.2 | 9.1-9.3 | 161 | 1221.0 | 1080-1310 |
| 4 | 17.0 | 16.9-17.2 | 8.50 | 8.45-8.55 | 9.2 | 9.0-9.3 | 161 | 536.8 | 505- 555 |
| 5 | 17.0 | 16.9-17.2 | 8.51 | 8.50-8.55 | 9.2 | 9.0-9.4 | 161 | 393.6 | 360- 415 |
| 6 | 17.0 | 16.9-17.2 | 8.49 | 8.45-8.50 | 9.2 | 9.0-9.3 | 161 | 224.1 | 200- 240 |
| 7 (control) | 17.0 | 16.9-17.2 | 8.49 | 8.45-8.50 | 9.2 | 9.1-9.3 | 161 | 8.1 | 7.3- 8.6 |

[1]Measured once during the bioassay.

Table 12. Results of Test J1.

| Mean Potassium Concentration (mg/l) | Percent Dead at Hr 48 | Hr 72 | Hr 96 | Hr 120 | Hr 168 | Hr 240 | Hr 264 | Hr 293 | Hr 312 |
|---|---|---|---|---|---|---|---|---|---|
| 3532.0 | 66.7 | 88.9 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2103.0 | 10.0 | 40.0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 1221.0 | 10.0 | 30.0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 536.8 | 10.0 | 20.0 | 30 | 60 | 90 (88)[1] | 100 | 100 | 100 | 100 |
| 393.6 | 0 | 10.0 | 30 | 30 | 80 (75) | 80 (75) | 90 (87.5) | 90 (87.5) | 90 (87.5) |
| 224.1 | 20.0 | 20.0 | 30 | 30 | 40 (25) | 40 (25) | 60 (50) | 60 (50) | 60 (50) |
| 7.3 (control) | 0 | 0 | 0 | 0 | 20 (0) | 20 (0) | 20 (0) | 20 (0) | 20 (0) |
| LC50 | 2700 | 1680 | 520 | 435 | 300 | 270 | 250 | 250 | 250 |
| 95 percent confidence limits | 1753-4158 | 960-2940 | 366-738 | 292-648 | 225-399 | 213-343 | 189-330 | 189-330 | 189-330 |
| slope function | 1.63 | 1.88 | 1.49 | 2.23 | 1.59 | 1.47 | 1.37 | 1.37 | 1.37 |

[1]Numbers in parentheses indicate values corrected for control mortality.

Table 13. Test Conditions for Juvenile Test J2.

| Test Chamber | Temperature (C) Mean | Range | pH Mean | Range | Dissolved Oxygen (mg/l) Mean | Range | Total Alkalinity (mg/l as $CaCO_3$) Mean | Range | Potassium (mg/l) Mean | Range |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 16.6 | 15.8-17.0 | 8.11 | 8.10-8.12 | 9.3 | 9.1-9.5 | 140 | 128-152 | 3244.0 | 3236.0-3252.0 |
| 2 | 16.7 | 15.7-16.8 | 8.21 | 8.13-8.28 | 9.3 | 9.1-9.5 | 155 | 152-158 | 2062.0 | 2000.0-2124.0 |
| 3 | 16.5 | 15.6-16.9 | 8.26 | 8.18-8.35 | 9.3 | 9.1-9.6 | 157 | 156-160 | 1101.0 | 1100.0-1102.0 |
| 4 | 16.6 | 15.7-16.9 | 8.26 | 8.18-8.35 | 9.4 | 9.0-9.6 | 157 | 156-161 | 613.0 | 599.0- 621.0 |
| 5 | 16.8 | 15.9-17.0 | 8.26 | 8.20-8.37 | 9.3 | 9.0-9.5 | 154 | 152-156 | 361.3 | 352.0- 371.0 |
| 6 | 16.8 | 16.0-17.1 | 8.28 | 8.18-8.35 | 9.3 | 9.0-9.5 | 156 | 154-158 | 196.3 | 175.8- 209.2 |
| 7 (control) | 16.8 | 16.0-17.1 | 8.31 | 8.25-8.35 | 9.2 | 9.0-9.4 | 159 | 156-160 | 2.8 | 2.1- 3.5 |

Table 14. Results of Test J2.

| Mean Potassium Concentration (mg/l) | Percent Dead at Hr 48 | Hr 72 | Hr 96 | Hr 144 | Hr 192 | Hr 240 | Hr 312 | Hr 384 |
|---|---|---|---|---|---|---|---|---|
| 3244.0 | 55 | 95 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2062.0 | 5 | 45 | 90 (89.5)[1] | 100 | 100 | 100 | 100 | 100 |
| 1101.0 | 10 | 10 | 45 (42.1) | 95 (94.7) | 100 | 100 | 100 | 100 |
| 613.0 | 5 | 10 | 25 (21.1) | 75 (73.7) | 95 (94.4) | 100 | 100 | 100 |
| 361.3 | 15 | 20 | 25 (21.1) | 30 (26.3) | 60 (55.6) | 75 (70.6) | 85 (82.4) | 90 (87.5) |
| 196.3 | 0 | 0 | 0 (0) | 0 (0) | 10 (0) | 10 (0) | 10 (0) | 10 (0) |
| 2.8 (control) | 0 | 0 | 5 (0) | 5 (0) | 10 (0) | 15 (0) | 15 (0) | 20 (0) |
| LC50 | 3150[2] | 1960 | 1000 | 510 | 350 | 320 | 310 | 300 |
| 95 percent confidence limits | | 1574-2440 | 775-1290 | 419-621 | 280-437 | 283-362 | 271-355 | 261-345 |
| slope function | | 1.42 | 2.04 | 1.57 | 1.43 | 1.22 | 1.24 | 1.26 |

[1] Numbers in parentheses indicate values corrected for control mortality.

[2] Chi-square could not be determined.

Table 15. Test Conditions for Juvenile Test J3.

| Test Chamber | Temperature (C) Mean | Range | pH Mean | Range | Dissolved Oxygen (mg/l) Mean | Range | Total Alkalinity (mg/l as $CaCO_3$) Mean | Range | Potassium (mg/l) Mean | Range |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 16.8 | 16.6-17.3 | 8.64 | 8.60-8.68 | 9.3 | 8.9- 9.6 | 234 | 228-238 | 3166.7 | 3072.0-3248.0 |
| 2 | 16.9 | 16.6-17.3 | 8.65 | 8.61-8.67 | 9.3 | 8.8- 9.7 | 242 | 232-251 | 1909.3 | 1832.0-1980.0 |
| 3 | 16.9 | 16.6-17.3 | 8.66 | 8.61-8.69 | 9.4 | 8.8- 9.8 | 239 | 216-255 | 1022.3 | 960.0-1076.0 |
| 4 | 16.9 | 16.6-17.2 | 8.65 | 8.60-8.69 | 9.5 | 8.8- 9.9 | 250 | 204-255 | 557.8 | 515.0- 600.0 |
| 5 | 16.8 | 16.6-17.1 | 8.65 | 8.60-8.68 | 9.5 | 8.7-10.0 | 219 | 188-251 | 332.0 | 308.0- 352.0 |
| 6 | 16.8 | 16.5-17.1 | 8.65 | 8.60-8.68 | 9.5 | 9.1- 9.8 | 221 | 196-248 | 181.3 | 160.8- 196.0 |
| 7 (control) | 16.7 | 16.4-17.0 | 8.66 | 8.62-8.70 | 9.5 | 8.8-10.2 | 240 | 216-259 | 4.7 | 2.4- 10.0 |

Table 16. Results of Test J3.

| Mean Potassium Concentration (mg/l) | Percent Dead at Hr 96 | Hr 120 | Hr 144 | Hr 168 | Hr 192 | Hr 240 | Hr 312 | Hr 384 | Hr 456 | Hr 528 | Hr 600 | Hr 696 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3166.7 | 70 (64.7)[1] | 90 (88.2) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 1909.3 | 40 (29.4) | 60 (52.9) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 1022.3 | 25 (11.8) | 30 (17.6) | 50 (41.2) | 65 (58.8) | 70 (64.7) | 80 (75) | 95 (93.8) | 100 | 100 | 100 | 100 | 100 |
| 557.8 | 15 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 30 (12.5) | 55 (38.1) | 70 (58.8) | 95 (93.1) | 95 (93.1) | 95 (91.9) |
| 332.0 | 10 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 20 ( 5.9) | 20 ( 5.9) | 25 ( 6.3) | 25 ( 6.3) | 30 ( 3.8) | 45 (24.0) | 55 (38.1) | 65 (51.9) | 75 (59.5) |
| 181.3 | 10 ( 0 ) | 20 ( 5.9) | 20 ( 5.9) | 20 ( 5.9) | 20 ( 5.9) | 20 ( 0 ) | 22.2 ( 2.8) | 22.2 ( 0 ) | 22.2 ( 0 ) | 22.2 ( 0 ) | 33.3 ( 8.3) | 33.3 ( 0 ) |
| 4.7 (control) | 15 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 20 ( 0 ) | 20 ( 0 ) | 27.25 ( 0 ) | 27.25 ( 0 ) | 27.25 ( 0 ) | 27.25 ( 0 ) | 38.35 ( 0 ) |
| LC50 | 2500 | 1800 | 1070 | 980 | 925 | 850 | 685 | 560 | 480 | 430 | 320 | 320 |
| 95 percent confidence limits | 1678- 3725 | 1118- 2898 | 931- 1231 | 845- 1137 | 784- 1092 | 708- 1020 | 543- 863 | 475- 661 | 403- 571 | 364- 407 | 258- 397 | 260- 394 |
| slope function | 1.89 | 1.72 | 1.26 | 1.27 | 1.31 | 1.34 | 1.46 | 1.31 | 1.48 | 1.30 | 1.42 | 1.40 |

[1]Numbers in parentheses indicate values corrected for control mortality.

Table 17. Test Conditions for Juvenile Test J4.

| Test Chamber | Temperature (C) Mean | Range | pH Mean | Range | Dissolved Oxygen (mg/l) Mean | Range | Total Alkalinity (mg/l as $CaCO_3$) Mean | Range | Potassium (mg/l) Mean | Range |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.4 | 6.0-6.9 | 8.37 | 8.35-8.38 | 11.6 | 11.2-12.2 | 207 | 206-208 | 3214.0 | 3196.0-3232.0 |
| 2 | 6.5 | 6.2-7.0 | 8.41 | 8.40-8.41 | 11.6 | 11.0-12.2 | 213 | 208-220 | 1894.0 | 1876.0-1912.0 |
| 3 | 6.6 | 6.0-7.0 | 8.42 | 8.41-8.42 | 11.5 | 11.0-11.8 | 212 | 210-214 | 1052.0 | 1046.0-1058.0 |
| 4 | 6.7 | 6.1-7.0 | 8.44 | 8.42-8.45 | 11.4 | 10.8-11.6 | 212 | 210-212 | 571.5 | 562.0- 581.0 |
| 5 | 6.7 | 5.2-6.8 | 8.46 | 8.40-8.55 | 11.5 | 11.0-11.8 | 204 | 200-208 | 348.7 | 341.0- 355.0 |
| 6 (control) | 6.5 | 5.2-7.1 | 8.41 | 8.32-8.49 | 11.2 | 11.0-12.0 | 218 | 212-220 | 9.2 | 2.4- 12.9 |

Table 18. Results of Test J4.

| Mean Potassium Concentration (mg/l) | Percent Dead at Hr 72 | Hr 96 | Hr 144 | Hr 192 | Hr 240 | Hr 312 | Hr 384 | Hr 456 | Hr 552 | Hr 648 |
|---|---|---|---|---|---|---|---|---|---|---|
| 3214 | 60 (57.9)[1] | 85 (84.2) | 95 (94.4) | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 1894 | 40 (36.8) | 55 (52.6) | 95 (94.4) | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 1052 | 10 ( 5.3) | 30 (26.3) | 50 (44.4) | 60 (55.6) | 85 (83.3) | 100 | 100 | 100 | 100 | 100 |
| 571.5 | 0 ( 0 ) | 0 ( 0 ) | 0 ( 0 ) | 10 ( 0 ) | 25 (16.7) | 45 (35.3) | 70 (64.7) | 2 | 2 | 2 |
| 348.7 | 10 ( 5.3) | 10 ( 5.3) | 25 (16.7) | 25 (16.7) | 30 (22.2) | 35 (23.5) | 35 (23.5) | 40 (29.4) | 45 (35.3) | 50 (41.2) |
| 9.2 (control) | 5 ( 0 ) | 5 ( 0 ) | 10 ( 0 ) | 10 ( 0 ) | 10 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) | 15 ( 0 ) |
| LC50 | 2700 | 1825 | 1180 | 1020 | 840 | 540 | 495 | 440 | 400 | 388 |
| 95 percent confidence limits | 2046-3564 | 1313-2537 | 1009-1381 | 894-1164 | 709-996 | 406-718 | 410-497 | 344-563 | 310-516 | 301-500 |
| slope function | 1.89 | 1.70 | 1.29 | 1.24 | 1.32 | 1.61 | 1.36 | 1.48 | 1.52 | 1.52 |

[1] Numbers in parentheses indicate values corrected for control mortality.

[2] Test chamber contaminated.

Table 19. Test Conditions for Chronic Bioassay K1.

| Test Chamber | Temperature (C) | Dissolved Oxygen mg/l | Dissolved Oxygen % Saturation | pH | Total Alkalinity mg/l $CaCO_3$ | Potassium mg/l K |
|---|---|---|---|---|---|---|
| 6 | 22.9 ± 0.81 [1] (21.4-24.4) | 7.19 ± 0.391 (6.30-8.30) | 84.7 ± 3.97 (75.7-97.4) | 8.03 ± 0.063 (7.90-8.14) | 169 ± 2.3 (164-172) | 195 ± 12.73 (186-205) |
| 5 | 22.9 ± 0.78 (21.6-24.3) | 7.36 ± 0.294 (6.65-7.90) | 86.7 ± 3.26 (77.7-93.7) | 8.02 ± 0.063 (7.89-8.11) | 170 ± 3.3 (164-176) | 115 [2] |
| 4 | 23.0 ± 0.80 (21.6-24.4) | 7.28 ± 0.290 (6.64-7.85) | 85.8 ± 2.61 (79.8-91.3) | 8.02 ± 0.054 (7.90-8.09) | 170 ± 2.4 (167-176) | 69.5 ± 2.12 (68.0-71.0) |
| 3 | 23.0 ± 0.82 (21.6-24.4) | 7.38 ± 0.310 (6.52-7.90) | 87.1 ± 2.92 (78.4-91.5) | 8.03 ± 0.057 (7.90-8.13) | 169 ± 1.7 (168-172) | 43.6 ± 0.07 (43.5-43.6) |
| 2 | 23.0 ± 0.85 (21.4-24.4) | 7.29 ± 0.306 (6.55-7.95) | 86.0 ± 2.74 (78.7-91.2) | 8.03 ± 0.056 (7.90-8.12) | 170 ± 2.4 (166-175) | 30.5 [2] |
| 1 (control) | 23.0 ± 0.86 (21.4-24.4) | 6.95 ± 0.540 (6.08-8.04) | 82.0 ± 5.57 (72.4-92.2) | 7.97 ± 0.068 (7.89-8.15) | 169 ± 2.2 (168-175) | 11.9 ± 1.59 (10.8-13.0) |

[1] Mean ± standard deviation.
[2] (Range)
Only one sample analyzed.

Table 20. Results of Chronic Bioassay K1.

| Test Chamber | Potassium Concentration mg/l | | Days of Exposure 0 | 14 | 28 | 42 |
|---|---|---|---|---|---|---|
| 6 | 195 ± 12.7 (186-204) | Mean Length mm | 2.6 ± 0.36 [1] (1.9-3.2) | 3.4 ± 0.55 (1.9-4.8) | 3.6 ± 0.59 (1.9-5.0) | 3.7 ± 0.60 (1.9-5.1) |
| | | Total Mortality % | - | 7.5 | 15 | 41.3 |
| 5 | 115 [2] | Mean Length mm | 2.6 ± 0.34 (1.8-3.1) | 3.5 ± 0.56 (2.3-4.9) | 3.8 ± 0.58 (2.8-5.3) | 4.1 ± 0.57 (3.1-5.2) |
| | | Total Mortality % | - | 10.4 | 15.6 | 48.8 |
| 4 | 69.5 ± 2.12 (68.0-71.0) | Mean Length mm | 2.5 ± 0.35 (2.0-3.5) | 3.4 ± 0.51 (2.5-4.9) | 3.5 ± 0.52 (2.6-4.9) | 3.7 ± 0.63 (2.7-5.1) |
| | | Total Mortality % | - | 11.2 | 14.0 | 30.0 |
| 3 | 43.6 ± 0.07 (43.5-43.6) | Mean Length mm | 2.6 ± 0.30 [3] (1.8-3.0) | | | |
| | | Total Mortality % | - | | | |
| 2 | 30.5 [2] | Mean Length mm | 2.5 ± 0.33 (1.9-3.2) | 3.5 ± 0.51 (2.6-4.3) | 3.6 ± 0.48 (2.7-4.6) | 3.7 ± 0.55 (2.7-4.5) |
| | | Total Mortality % | - | 0 | 5.0 | 30.0 |
| 1 | 11.9 ± 1.59 (10.8-13.0) | Mean Length mm | 2.6 ± 0.28 (2.1-3.1) | 3.4 ± 0.40 (2.6-4.0) | 3.5 ± 0.39 (2.6-4.1) | 3.7 ± 0.36 (3.0-4.2) |
| | | Total Mortality % | - | 10.5 | 18.5 | 67.4 |

[1] Mean ± standard deviation (Range).
[2] Only one sample analyzed.
[3] Tank contaminated, eliminated from test.

Table 21. Test Conditions for Chronic Bioassay K2.

| Test Chamber | Temperature (C) | Dissolved Oxygen mg/l | Dissolved Oxygen % Saturation | pH | Total Alkalinity mg/l $CaCO_3$ | Potassium mg/l K |
|---|---|---|---|---|---|---|
| 6 | 23.9 ± 0.71[1] (21.8-24.7) | 7.60 ± 0.496 (6.49-8.70) | 91.3 ± 5.78 (76.1-100.6) | 8.14 ± 0.083 (7.92-8.29) | 167 ± 3.6 (162-172) | 275[2] |
| 5 | 24.0 ± 0.74 (21.7-24.7) | 7.69 ± 0.493 (5.99-8.64) | 92.4 ± 5.95 (70.1-101.7) | 8.14 ± 0.083 (7.91-8.28) | 170 ± 4.4 (164-176) | 184 |
| 4 | 24.0 ± 0.73 (21.7-24.7) | 7.67 ± 0.470 (6.20-8.62) | 92.1 ± 5.61 (72.6-100.4) | 8.13 ± 0.085 (7.90-8.29) | 168 ± 4.5 (162-174) | 106 |
| 3 | 23.9 ± 0.72 (21.7-24.7) | 7.71 ± 0.504 (6.07-8.80) | 92.7 ± 6.07 (71.1-101.6) | 8.14 ± 0.094 (7.88-8.29) | 167 ± 3.7 (163-173) | 65 |
| 2 | 23.9 ± 0.72 (21.7-24.7) | 7.68 ± 0.486 (6.20-8.74) | 92.1 ± 5.76 (72.7-101.6) | 8.14 ± 0.088 (7.89-8.29) | 168 ± 5.1 (162-176) | 45 |
| 1 (control) | 23.8 ± 0.67 (21.8-24.5) | 7.74 ± 0.465 (6.55-8.70) | 92.7 ± 5.40 (76.8-100.6) | 8.12 ± 0.094 (7.89-8.29) | 167 ± 4.4 (162-172) | 14.3 |

[1] Mean ± standard deviation (Range).
[2] Only one sample analyzed.

Table 22. Results of Chronic Bioassay K2.

| Test Chamber | Potassium Concentration mg/l | | Days of Exposure 0 | 14 | 28 |
|---|---|---|---|---|---|
| 6 | 275[1] | Mean Length mm | 2.5 ± 0.27[2] (1.9-3.2) | 2.6 ± 0.28 (2.2-3.3) | 2.7 ± 0.45 (2.0-3.3) |
| | | Total Mortality % | - | 40 | 84.2 |
| 5 | 184 | Mean Length mm | 2.6 ± 0.25 (2.1-3.2) | 3.1 ± 0.40 (2.3-3.9) | 3.4 ± 0.48 (2.3-4.5) |
| | | Total Mortality % | - | 5.5 | 13.2 |
| 4 | 106 | Mean Length mm | 2.5 ± 0.23 (2.1-3.1) | 3.2 ± 0.41 (2.2-4.1) | 3.9 ± 0.59 (2.3-4.9) |
| | | Total Mortality % | - | 2.5 | 12.8 |
| 3 | 65 | Mean Length | 2.6 ± 0.27 (2.1-3.2) | 3.3 ± 0.47 (2.3-4.5) | 3.4 ± 0.48 (2.4-4.5) |
| | | Total Mortality % | - | 2.6 | 10.3 |
| 2 | 45 | Mean Length mm | 2.5 ± 0.23 (2.2-3.0) | 3.0 ± 0.33 (2.3-3.7) | 3.3 ± 0.40 (2.6-4.2) |
| | | Total Mortality % | - | 5.0 | 37.5 |
| 1 (control) | 14 | Mean Length | 2.6 ± 0.26 (2.2-3.2) | 3.0 ± 0.29 (2.2-3.5) | 3.1 ± 0.25 (2.6-3.4) |
| | | Total Mortality % | - | 5.2 | 43.6 |

[1] Only one sample analyzed.
[2] Mean ± standard deviation (Range).

Table 23. Test Conditions for Chronic Bioassay $NH_3$2.

| Test Chamber | Temperature (C) | Dissolved Oxygen mg/l | Dissolved Oxygen % Saturation | pH | Total Alkalinity mg/l $CaCO_3$ | Free $CO_2$ mg/l | Total Ammonia mg/l $NH_3$-N | Undissociated Ammonia mg/l $NH_3$-N |
|---|---|---|---|---|---|---|---|---|
| 6 | 23.5 ± 0.45[1] (22.9-24.4) | 6.75 ± 0.728 (5.02-7.95) | 80.3 ± 8.50 (59.6-94.0) | 8.09 ± 0.161 (7.88-8.55) | 165 ± 3.0 (160-168) | 3.12 ± 0.420 (2.46-3.73) | 16.16 ± 2.219 (12.84-19.88) | 0.93 ± 0.304 (0.46-1.53) |
| 5 | 23.5 ± 0.45 (22.8-24.4) | 7.20 ± 0.643 (5.91-8.20) | 85.6 ± 7.40 (70.0-96.5) | 8.15 ± 0.116 (7.92-8.49) | 168 ± 3.1 (163-172) | 2.40 ± 0.280 (2.12 ± 2.94) | 8.88 ± 1.514 (5.88-11.27) | 0.59 ± 0.144 (0.34-0.82) |
| 4 | 23.5 ± 0.47 (22.9-24.4) | 7.38 ± 0.567 (6.00-8.19) | 87.8 ± 6.32 (72.6-96.2) | 8.16 ± 0.099 (7.94-8.37) | 168 ± 2.1 (163-171) | 2.40 ± 0.410 (1.98-3.31) | 5.07 ± 0.909 (2.89-6.20) | 0.35 ± 0.089 (0.17-0.49) |
| 3 | 23.4 ± 0.46 (22.2-24.4) | 7.44 ± 0.527 (6.24-8.20) | 88.5 ± 5.80 (75.4-96.4) | 8.14 ± 0.071 (7.96-8.35) | 168 ± 1.6 (166-171) | 2.42 ± 0.250 (1.96-2.80) | 3.03 ± 0.575 (1.80-3.78) | 0.20 ± 0.051 (0.11-0.27) |
| 2 | 23.4 ± 0.47 (22.8-24.4) | 7.58 ± 0.424 (6.90-8.28) | 90.1 ± 4.68 (82.3-97.2) | 8.16 ± 0.055 (8.04-8.30) | 168 ± 1.9 (167-172) | 2.29 ± 0.194 (1.96-2.60) | 1.48 ± 0.326 (0.82-1.92) | 0.10 ± 0.030 (0.05-0.13) |
| 1 (control) | 23.5 ± 0.47 (22.8-24.5) | 7.84 ± 0.315 (7.45-8.52) | 92.9 ± 3.02 (87.3-97.4) | 8.20 ± 0.041 (8.13-8.33) | 169 ± 2.2 (168-172) | 1.99 ± 0.185 (1.54-2.15) | 0.10 ± 0.026 (0.06-0.14) | 0.01 ± 0.002 (0.004-0.01) |

[1]Mean ± standard deviation (Range).

Table 24. Results of Chronic Bioassay $NH_3$2.

| Test Chamber | | Days of Exposure 0 | 28 | 42 |
|---|---|---|---|---|
| 6 | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 1.01 ± 0.300 (0.64-1.53) | 0.93 ± 0.304 (0.46-1.53) |
| | Mean Length mm | 2.3 ± 0.28[1] (1.7-2.9) | 2.3 ± 0.25 (1.9-2.7) | 2.3 ± 0.31 (2.0-2.7) |
| | Total Mortality % | - | 49.5 | 92.2 |
| 5 | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 0.62 ± 0.143 (0.45-0.82) | 0.59 ± 0.144 (0.34-0.82) |
| | Mean Length mm | 2.3 ± 0.28 (1.8-2.8) | 2.3 ± 0.27 (1.9-2.9) | 2.2 ± 0.29 (1.8-2.9) |
| | Total Mortality % | - | 28.7 | 66.6 |
| 4 | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 0.34 ± 0.085 (0.17-0.43) | 0.35 ± 0.089 (0.17-0.49) |
| | Mean Length mm | 2.2 ± 0.19 (1.8-2.7) | 2.3 ± 0.20 (1.8-2.7) | 2.3 ± 0.20 (2.0-2.7) |
| | Total Mortality % | - | 7.9 | 21.6 |
| 3 | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 0.21 ± 0.510 (0.11-0.27) | 0.20 ± 0.051 (0.11-0.27) |
| | Mean Length mm | 2.2 + 0.24 (1.8-2.7) | 2.4 + 0.23 (1.9-2.8) | 2.4 + 0.26 (1.9-2.9) |
| | Total Mortality % | - | 6.3 | 10.0 |
| 2 | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 0.10 ± 0.031 (0.05-0.13) | 0.10 ± 0.030 (0.05-0.13) |
| | Mean Length mm | 2.1 ± 0.20 (1.8-2.6) | 2.4 ± 0.27 (1.8-3.0) | 2.6 ± 0.32 (1.8-3.3) |
| | Total Mortality % | - | 7.5 | 13.2 |
| 1 (control) | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 0.01 ± 0.002 (0.005-0.01) | 0.01 ± 0.002 (0.004-0.01) |
| | Mean Length mm | 2.2 ± 0.32 (1.8-2.8) | 2.3 ± 0.33 (1.8-3.1) | 2.3 ± 0.32 (1.8-3.1) |
| | Total Mortality % | - | 15.0 | 21.6 |

[1]Mean ± standard deviation (Range).

Table 25. Test Conditions for Chronic Bioassay $NH_3$3.

| Test Chamber | Temperature (C) | Dissolved Oxygen mg/l | Dissolved Oxygen % Saturation | pH | Total Alkalinity mg/l $CaCO_3$ | Free $CO_2$ mg/l[2] | Total Ammonia mg/l $NH_3$-N | Undissociated Ammonia mg/l $NH_3$-N |
|---|---|---|---|---|---|---|---|---|
| 6 | 23.0 ± 0.24[1] | 6.55 ± 0.768 | 77.1 ± 9.14 | 8.14 ± 0.072 | 166 ± 4.1 | 2.27 ± 0.387 | 18.04 ± 0.650 | 1.20 ± 0.197 |
| | (22.2-23.4) | (5.35-8.40) | (63.5-99.3) | (7.98-8.30) | (158-171) | (1.96-3.07) | (16.1-18.91) | (0.84-1.53) |
| 5 | 22.9 ± 0.28 | 6.82 ± 0.716 | 80.2 ± 8.61 | 8.12 ± 0.061 | 166 ± 5.0 | 2.32 ± 0.310 | 9.51 ± 0.516 | 0.60 ± 0.097 |
| | (22.0-23.4) | (5.70-8.48) | (66.9-100.2) | (7.99-8.28) | (158-173) | (1.82-2.76) | (8.33-10.17) | (0.43-0.74) |
| 4 | 22.9 ± 0.26 | 7.12 ± 0.658 | 83.9 ± 7.89 | 8.10 ± 0.065 | 156 ± 5.4 | 2.35 ± 0.350 | 5.51 ± 0.288 | 0.34 ± 0.062 |
| | (22.2-23.4) | (5.80-8.50) | (68.1-100.8) | (7.98-8.29) | (158-172) | (1.77-2.81) | (4.79-5.87) | (0.27-0.46) |
| 3 | 22.9 ± 0.24 | 7.14 ± 0.649 | 84.1 ± 7.79 | 8.08 ± 0.066 | 164 ± 5.5 | 2.43 ± 0.410 | 3.33 ± 0.211 | 0.20 ± 0.040 |
| | (22.2-23.3) | (6.20-8.55) | (72.7-101.3) | (7.97-8.28) | (159-172) | (1.83-2.89) | (2.88-3.61) | (0.14-0.27) |
| 2 | 22.9 ± 0.27 | 7.34 ± 0.614 | 86.5 ± 7.35 | 8.09 ± 0.068 | 165 ± 5.8 | 2.40 ± 0.420 | 1.59 ± 0.159 | 0.10 ± 0.023 |
| | (22.1-23.3) | (6.30-8.59) | (75.2-101.8) | (7.98-8.28) | (158-173) | (1.79-2.85) | (1.29-1.80) | (0.07-0.14) |
| 1 (control) | 23.0 ± 0.32 | 8.11 ± 0.365 | 95.6 ± 4.53 | 8.18 ± 0.060 | 165 ± 3.8 | 2.06 ± 0.320 | 0.08 ± 0.022 | 0.01 ± 0.002 |
| | (22.0-23.3) | (7.50-8.80) | (88.2-104.3) | (8.03-8.30) | (161-171) | (1.74-2.71) | (0.04-0.13) | (0.003-0.01) |

[1] Mean ± standard deviation (Range).

Table 26. Results of Chronic Bioassay $NH_3$3.

| Test Chamber | | Days of Exposure 0 | 14 | 28 | 42 |
|---|---|---|---|---|---|
| 6 | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 1.17 ± 0.202 (0.84-1.32) | 1.21 ± 0.189 (0.84-1.53) | 1.20 ± 0.197 (0.84-1.53) |
| | Mean Length mm | 2.5 ± 0.20[1] (2.1-2.9) | 2.5 ± 0.19 (2.2-2.7) | 2.5 ± 0.15 (2.3-2.7) | - |
| | Total Mortality % | - | 33.9 | 86.7 | 100 |
| 5 | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 0.63 ± 0.126 (0.48-0.74) | 0.62 ± 0.097 (0.48-0.74) | 0.60 ± 0.097 (0.43-0.74) |
| | Mean Length mm | 2.5 ± 0.26 (2.1-3.1) | 2.5 ± 0.22 (2.1-3.1) | 2.5 ± 0.22 (2.1-3.1) | 2.5 ± 0.12 (2.2-2.6) |
| | Total Mortality % | - | 10 | 25 | 71.7 |
| 4 | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 0.38 ± 0.076 (0.28-0.46) | 0.35 ± 0.066 (0.28-0.46) | 0.34 ± 0.062 (0.27-0.46) |
| | Mean Length mm | 2.4 ± 0.19 (2.1-2.8) | 2.5 ± 0.19 (2.1-2.9) | 2.5 ± 0.20 (2.1-2.9) | 2.6 ± 0.20 (2.1-3.0) |
| | Total Mortality % | - | 2.5 | 12.8 | 26.8 |
| 3 | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 0.22 ± 0.036 (0.17-0.27) | 0.20 ± 0.037 (0.16-0.27) | 0.20 ± 0.040 (0.14-0.27) |
| | Mean Length mm | 2.5 ± 0.22 (2.0-3.0) | 2.7 ± 0.32 (2.1-3.4) | 3.0 ± 0.41 (2.2-4.4) | 3.3 ± 0.43 (2.6-4.5) |
| | Total Mortality % | - | 5 | 7.5 | 18.5 |
| 2 | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 0.12 ± 0.018 (0.10-0.14) | 0.10 ± 0.026 (0.07-0.14) | 0.10 ± 0.023 (0.07-0.14) |
| | Mean Length mm | 2.5 ± 0.26 (1.9-3.1) | 3.1 ± 0.45 (2.3-4.1) | 3.5 ± 0.53 (2.7-4.8) | 3.9 ± 0.62 (2.9-5.2) |
| | Total Mortality % | - | 0 | 2.5 | 7.8 |
| 1 (control) | Interval Undissociated Ammonia mg/l $NH_3$-N | - | 0.01 ± 0.002 (0.004-0.01) | 0.01 ± 0.002 (0.004-0.01) | 0.01 ± 0.002 (0.003-0.01) |
| | Mean Length mm | 2.6 ± 0.21 (2.2-2.8) | 2.7 ± 0.26 (2.2-3.2) | 2.9 ± 0.33 (2.4-3.7) | 3.0 ± 0.40 (2.4-4.1) |
| | Total Mortality % | - | 10 | 18.9 | 26.7 |

[1] Mean ± standard deviation (Range).

## APPENDIX B. PUBLICATIONS AND THESIS RESULTING FROM THIS RESEARCH.

### Publications

Anderson, K.B., M.J. Sandusky, and R.E. Sparks. 1977. The toxicity of potassium, undissociated ammonia and Illinois River water to the fingernail clam (Musculium transversum). Abstracts and program of the 39th Midwest Fish and Wildlife Conference: 35-36 (abstract).

Anderson, K.B., C.M. Thompson, R.E. Sparks, and A.A. Paparo. 1976. Effects of potassium on adult Asiatic clams, Corbicula manilensis. Biological Notes No. 98. Illinois Natural History Survey. Urbana. 7 p.

Anonymous. 1977. Of clams and ducks. The Illinois Natural History Survey Reports No. 164: 1-2. February.

Murphy, J.A. and A.A. Paparo. 1976. Cytosomal and neuronal changes during photoreception as related to ionic permeability and ciliary activity in Mytilus edulis, p. 200-201. In: G.W. Bailey (ed.), 34th Ann. Proc. Electron Microscopy Soc. Amer., Miami Beach, Florida.

Paparo, A.A. 1976. The coordinate roles of branchial nerve activity and potassium in the stimulation of ciliary activity in Mytilus edulis: observations with phenoxybenzamine, bromolysergic acid and fluorescence histochemistry. J. Exp. Biol. 65: 109-116.

Paparo, A.A. and J.A. Murphy. 1976. Light-dark changes in the morphology and elemental composition of pigment granules in the nerve ending under the lateral ciliated cell of the mussel, Mytilus edulis, p. 577-584. In: Scanning Electron Microscopy/1976 (Part VIII), Proceedings of the Workshop on Zoological Applications of SEM. IIT Research Institute. Chicago.

Paparo, A.A., K. Cunningham-Paparo, and J. Murphy. 1977. DOPA decarboxylase activities and potassium stimulation of lateral cilia on the gill of Mytilus edulis. I. A response to DOPA decarboxylase inhibitors and chemical sympathectomy. Bulletin of the Southern California Academy of Sciences 76(1): 32-37.

Paparo, A.A., K. Cunningham-Paparo, and J. Murphy. 1977. The effect of endogenous 5-HT on K ion enhancement of ciliary activity in the mussel Mytilus edulis. Bulletin of the Southern California Academy of Sciences 76(2): 111-115.

Paparo, A.A. and R.E. Sparks. 1977. Rapid assessment of water quality using the fingernail clam, Musculium transversum, p. 96-109. In: J. Cairns, Jr., K.L. Dickson, and G.F. Westlake (eds.), Biological Monitoring of Water and Effluent Quality. American Society for Testing and Materials Special Publication 607. Philadelphia.

Paparo, A.A., R.E. Sparks, J.A. Murphy, and K.J. Cunningham-Paparo. 1977. The effect of potassium ions on the rate of ciliary activity in Sphaerium transversum. I. A different response in small and large clam preparations. Bulletin of the Southern California Academy of Sciences 76(3): 139-145.

Sparks, R.E. and K.B. Anderson. 1977. Assessing toxicities in surface waters, with emphasis on the Illinois River. Abstracts with programs, North Central Section, Eleventh Annual Meeting, The Geological Society of America 9(5): 653 (abstract).

Thompson, C.M. and R.E. Sparks. 1977. Status of the fingernail clam (Musculium transversum) in the Keokuk Pool, Mississippi River. Abstracts and program of the 39th Midwest Fish and Wildlife Conference: 24 (abstract).

Thesis

Anderson, K.B. 1977. Musculium transversum in the Illinois River and an acute potassium bioassay method for the species. M.S. thesis. Western Illinois University. Macomb. 79 p.

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